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Washington DC 20231

Date May 26, 1998

ASSISTANT COMMISSIONER OF PATENTS AND TRADEMARKS
Washington DC 20231

Transmitted herewith for filing is the patent application of
Inventors: Heinrich D. LÜTTICKEN, Egbert MUNDT and Adriaan A.W.M. LOON

For: RECOMBINANT BIRNAVIRUS VACCINE

- [X] Specification and claims (61 pages)
[X] Five (5) sheets of drawings.
[] An assignment of the invention to _____
[X] Sequence Listing (Paper and CRF Disk)
[X] Preliminary Amendment
[X] Information Disclosure Statement/PTO Form 1449/References
[X] A filing fee calculated as shown below:

FOR:	NO. FILED	NO. EXTRA	RATE	FEE
BASIC FEE				\$ 790.00
TOTAL CLAIMS	29-20 =	9	X \$ 22	\$ 198.00
INDEP CLAIMS	3- 3 =	0	X \$ 82	\$.00
[X] MULTIPLE DEPENDENT CLAIMS PRESENTED			+ \$270	\$ 270.00
			TOTAL	\$1258.00

[X] Please charge my Deposit Account No. 02-2334 in the amount of \$1258.00 to cover the filing fee and [] assignment recordation.

[X] The Commissioner is hereby authorized to charge payment for the following fees associated with this communication or credit any overpayment to Deposit Account No. 02-2334.

[X] Any additional filing fees required under 37 CFR 1.16.

[X] The Commissioner is hereby authorized to charge payment for the following fees associated with this communication or credit any overpayment to Deposit Account No. 02-2334.

[X] Any patent application processing fees under 37 CFR 1.17.

[X] Any filing fees under 37 CFR 1.16 for presentation of extra claims.

Respectfully submitted,
AKZO NOBEL N.V.

Mary E. Gormley
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Enclosures

Attorney Docket No. I/97269 US

Express Mail No. EL042439813US
58lutckn.fil

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Heinrich D. LÜTTICKEN, Egbert MUNDT and Adriaan A.W.M. LOON

Serial Number: to be assigned Group Art Unit: to be assigned

Filed: Concurrently herewith Examiner: to be assigned

For: RECOMBINANT BIRNAVIRUS VACCINE

PRELIMINARY AMENDMENT AND SUBMISSION OF SEQUENCE LISTING

Assistant Commissioner of Patents
Washington, D.C. 20231

May 26, 1998

Sir:

Prior to the calculation of the fee in the above-identified application, please make the following amendments:

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 1, above line 4, insert -- Field of the Invention --; and
between lines 6 and 7, insert -- Background of the Invention --.

Page 3, line 14, insert -- Summary of the Invention --; and
line 21, insert -- Detailed Description of the Invention --.

Please delete pages 29 - 58 in their entireties and replace them with the attached Sequence Listing as pages 29 - 56.

Please renumber pages 59 - 61 as pages 57 - 59, respectively.

Express Mail No.
EL042439813US

-1-

IN THE CLAIMS:

Please amend the claims as follows:

2. (amended) A birnavirus mutant according to claim 1, [characterised in that] wherein the mutation is a substitution.

3. (amended) A birnavirus mutant according to claim 1, [characterised in that] wherein the mutation is an insertion of a heterologous nucleic acid sequence.

4. (amended) A birnavirus mutant according to claim 3, [characterised in that] wherein the heterologous nucleic acid sequence encodes a polypeptide and the heterologous nucleic acid sequence is under the control of an expression control sequence regulating the expression of the sequence in a cell infected with the virus mutant.

5. (amended) A birnavirus mutant according to [claims 1-4, characterised in that] claim 1, wherein the birnavirus is infectious bursal disease virus (IBDV).

6. (amended) A birnavirus mutant according to claim 5, [characterised in that] wherein the mutation is in the genome of a virulent field virus.

7. (amended) A birnavirus mutant according to claim 5, [characterised in that] wherein the mutation is in the genome of a vaccine strain[, preferably in vaccine strain D78].

8. (amended) A birnavirus mutant according to [claims 5-7, characterised in that] claim 5, wherein the mutant has a mutated start codon and three stop codons in the 5'-end of the VP5 gene as shown in SEQ ID NO:7.

9. (amended) A birnavirus according to [claims 5-8, characterised in that] claim 5, wherein the IBDV expresses a chimeric VP2 protein comprising virus neutralizing epitopes of different antigenic IBDV types.

10. (amended) A vaccine against a birnavirus infection in animals, [characterised in that it comprises] comprising a birnavirus mutant according to any one of claims 1-9 and a pharmaceutically acceptable carrier.

Please cancel claim 11 without prejudice or disclaimer of the subject matter thereof.

12. (amended) A method [according to claim 11, characterised in that the method comprises] for determining birnavirus infection in an animal, comprising the steps of:

- (i) incubating a sample suspected of containing anti-birnavirus antibodies[,] with VP5 antigen,
- (ii) allowing the formation of antibody-antigen complex, and
- (iii) detecting the presence of the antibody-antigen complex,

wherein the presence of the complex indicates a birnavirus infection.

13. (amended) A diagnostic kit suitable for carrying out a method according to [claims 11-12] claim 12, comprising VP5 antigen coated on a solid phase.

Please cancel claim 14 without prejudice or disclaimer of the subject matter thereof.

Please add the following new claims 15 - 31.

-- 15. A birnavirus mutant according to claim 7, wherein the vaccine strain is D78. --

-- 16. A diagnostic test kit according to claim 13, further comprising an enzyme-conjugated antibody and substrate to said enzyme. --

-- 17. A method for determining birnavirus infection in an animal, comprising:

(i) incubating a sample suspected of containing VP5 with anti-birnavirus VP5 antibody;

(ii) allowing the formation of antibody-antigen complex; and

(iii) detecting the presence of antibody-antigen complex, wherein the presence of the complex indicates birnavirus infection. --

-- 18. A diagnostic test kit for carrying out a method according to claim 17, comprising a container having anti-birnavirus VP5 antibody. --

-- 19. A diagnostic test kit according to claim 18, further comprising a second labelled antibody which will detect said complex. --

-- 20. A diagnostic test kit according to claim 18, wherein the antibody is labelled. --

-- 21. A diagnostic test kit according to claim 18, wherein the antibody is coated on a solid phase. --

-- 22. A birnavirus according to claim 2, wherein the birnavirus is IBDV. --

-- 23. A birnavirus according to claim 3, wherein the birnavirus is IBDV. --

-- 24. A birnavirus according to claim 22, wherein the mutation is in the genome of a virulent field virus. --

-- 25. A birnavirus according to claim 23, wherein the mutation is in the genome of a virulent field virus. --

-- 26. A birnavirus according to claim 22, wherein the mutation is in the genome of a vaccine strain. --

-- 27. A birnavirus according to claim 23, wherein the mutation is in the genome of a vaccine strain. --

-- 28. A birnavirus according to claim 26, wherein the vaccine strain is D78. --

-- 29. A birnavirus according to claim 27, wherein the vaccine strain is D78. --

-- 30. A birnavirus according to claim 6, wherein the IBDV expresses a chimeric VP2 protein comprising virus neutralizing epitopes of different antigenic IBDV types. --

-- 31. A vaccine against a birnavirus infection in animals, comprising a birnavirus mutant according to any one of claims 22 - 30 and a pharmaceutically acceptable carrier. --

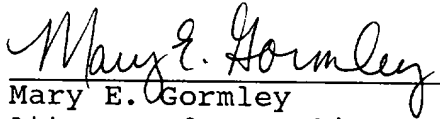
REMARKS

Claims 2 - 10, 12 and 13 are amended, claims 11 and 14 canceled, and claims 15 - 31 are added, hereby. Claims 1 - 10, 12, 13 and 15 - 31 are presented for examination.

Also submitted herewith is the Sequence Listing in both paper and CRF diskette. The name of the file on the diskette is 58LUTTIC.SEQ. The paper copy and CRF are the same and the sequences thereof are the same as in the original specification. No new matter has been added.

It is believed that claims 1 - 10, 12, 13 and 15 - 31 recite a patentable improvement in the art. Favorable action is solicited. In the event any fees are required with this paper, please charge our Deposit Account No. 02-2334.

Respectfully submitted,


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Attorney Docket I/97269 US

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Enclosure: Sequence Listing (Paper Copy and CRF)

58lutckn.pre

Express Mail No.
EL042439813US

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Lutticken, Heinrich D.
Mundt, Egbert
Loon, Adriaan A. W. M.
- (ii) TITLE OF INVENTION: Recombinant birnavirus vaccine
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Akzo Nobel Patent Dept.
 - (B) STREET: 1300 Piccard Drive, Suite 206
 - (C) CITY: Rockville
 - (D) STATE: Maryland
 - (E) COUNTRY: US
 - (F) ZIP: 20850
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30(EPO)
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 26-MAY-1998
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Gormley, Mary E.
 - (B) REGISTRATION NUMBER: 34,409
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 301-948-7400
 - (B) TELEFAX: 301-948-9751

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2827 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:112..2745

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGATACGATG GGTCTGACCC TCTGGGAGTC ACGAATTAAC GTGGCTACTA GGGGCGATAC	60
CCGCCGCTGG CCGCCACGTT AGTGGCTCCT CTTCTTGATG ATTCTGCCAC C ATG AGT	117
	Met Ser
	1
GAC ATT TTC AAC AGT CCA CAG GCG CGA AGC ACG ATC TCA GCA GCG TTC	165
Asp Ile Phe Asn Ser Pro Gln Ala Arg Ser Thr Ile Ser Ala Ala Phe	
	5 10 15
GGC ATA AAG CCT ACT GCT GGA CAA GAC GTG GAA GAA CTC TTG ATC CCT	213
Gly Ile Lys Pro Thr Ala Gly Gln Asp Val Glu Glu Leu Leu Ile Pro	
	20 25 30
AAA GTT TGG GTG CCA CCT GAG GAT CCG CTT GCC AGC CCT AGT CGA CTG	261
Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser Arg Leu	
	35 40 45 50
GCA AAG TTC CTC AGA GAG AAC GGC TAC AAA GTT TTG CAG CCA CGG TCT	309
Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Val Leu Gln Pro Arg Ser	
	55 60 65
CTG CCC GAG AAT GAG GAG TAT GAG ACC GAC CAA ATA CTC CCA GAC TTA	357
Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro Asp Leu	
	70 75 80
GCA TGG ATG CGA CAG ATA GAA GGG GCT GTT TTA AAA CCC ACT CTA TCT	405
Ala Trp Met Arg Gln Ile Glu Gly Ala Val Leu Lys Pro Thr Leu Ser	
	85 90 95
CTC CCT ATT GGA GAT CAG GAG TAC TTC CCA AAG TAC TAC CCA ACA CAT	453
Leu Pro Ile Gly Asp Gln Glu Tyr Phe Pro Lys Tyr Tyr Pro Thr His	
	100 105 110
CGC CCT AGC AAG GAG AAG CCC AAT GCG TAC CCG CCA GAC ATC GCA CTA	501
Arg Pro Ser Lys Glu Lys Pro Asn Ala Tyr Pro Pro Asp Ile Ala Leu	
	115 120 125 130
CTC AAG CAG ATG ATT TAC CTG TTT CTC CAG GTT CCA GAG GCC AAC GAG	549
Leu Lys Gln Met Ile Tyr Leu Phe Leu Gln Val Pro Glu Ala Asn Glu	
	135 140 145

GGC Gly	CTA Leu	AAG Lys	GAT Asp 150	GAA Glu	GTA Val	ACC Thr	CTC Leu	TTG Leu	ACC Thr	CAA Gln	AAC Asn	ATA Ile	AGG Arg 160	GAC Asp	AAG Lys	597
GCC Ala	TAT Tyr	GGA Gly 165	AGT Ser	GGG Gly	ACC Thr	TAC Tyr	ATG Met 170	GGA Gly	CAA Gln	GCA Ala	AAT Asn	CGA Arg 175	CTT Leu	GTG Val	GCC Ala	645
ATG Met	AAG Lys 180	GAG Glu	GTC Val	GCC Ala	ACT Thr	GGA Gly 185	AGA Arg	AAC Asn	CCA Pro	AAC Asn	AAG Lys 190	GAT Asp	CCT Pro	CTA Leu	AAG Lys	693
CTT Leu 195	GGG Gly	TAC Tyr	ACT Thr	TTT Phe	GAG Glu 200	AGC Ser	ATC Ile	GCG Ala	CAG Gln	CTA Leu 205	CTT Leu	GAC Asp	ATC Ile	ACA Thr	CTA Leu 210	741
CCG Pro	GTA Val	GGC Gly	CCA Pro	CCC Pro 215	GGT Gly	GAG Glu	GAT Asp	GAC Asp	AAG Lys 220	CCC Pro	TGG Trp	GTG Val	CCA Pro	CTC Leu 225	ACA Thr	789
AGA Arg	GTG Val	CCG Pro	TCA Ser 230	CGG Arg	ATG Met	TTG Leu	GTG Val	CTG Leu 235	ACG Thr	GGA Gly	GAC Asp	GTA Val	GAT Asp 240	GGC Gly	GAC Asp	837
TTT Phe	GAG Glu	GTT Val 245	GAA Glu	GAT Asp	TAC Tyr	CTT Leu	CCC Pro 250	AAA Lys	ATC Ile	AAC Asn	CTC Leu	AAG Lys 255	TCA Ser	TCA Ser	AGT Ser	885
GGA Gly	CTA Leu 260	CCA Pro	TAT Tyr	GTA Val	GGT Gly	CGC Arg 265	ACC Thr	AAA Lys	GGA Gly	GAG Glu	ACA Thr	ATT Ile	GGC Gly	GAG Glu	ATG Met	933
ATA Ile 275	GCT Ala	ATC Ile	TCA Ser	AAC Asn	CAG Gln 280	TTT Phe	CTC Leu	AGA Arg	GAG Glu	CTA Leu 285	TCA Ser	ACA Thr	CTG Leu	TTG Leu	AAG Lys 290	981
CAA Gln	GGT Gly	GCA Ala	GGG Gly	ACA Thr 295	AAG Lys	GGG Gly	TCA Ser	AAC Asn	AAG Lys 300	AAG Lys	AAG Lys	CTA Leu	CTC Leu	AGC Ser 305	ATG Met	1029
TTA Leu	AGT Ser	GAC Asp	TAT Tyr 310	TGG Trp	TAC Tyr	TTA Leu	TCA Ser	TGC Cys 315	GGG Gly	CTT Leu	TTG Leu	TTT Phe	CCA Pro 320	AAG Lys	GCT Ala	1077
GAA Glu	AGG Arg	TAC Tyr 325	GAC Asp	AAA Lys	AGT Ser	ACA Thr	TGG Trp 330	CTC Leu	ACC Thr	AAG Lys	ACC Thr	CGG Arg 335	AAC Asn	ATA Ile	TGG Trp	1125
TCA Ser	GCT Ala 340	CCA Pro	TCC Ser	CCA Pro	ACA Thr	CAC His 345	CTC Leu	ATG Met	ATC Ile	TCT Ser	ATG Met	ATC Ile	ACC Thr	TGG Trp	CCC Pro	1173

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TCA Ser	CTC Leu	TAC Tyr	AAA Lys 375	TTC Phe	AAC Asn	CCG Pro	TTC Phe	AGA Arg	GGA Gly 380	GGG Gly	TTG Leu	AAC Asn	AGG Arg	ATC Ile 385	GTC Val	1269
GAG Glu	TGG Trp	ATA Ile	TTG Leu 390	GCC Ala	CCG Pro	GAA Glu	GAA Glu	CCC Pro 395	AAG Lys	GCT Ala	CTT Leu	GTA Val	TAT Tyr 400	GCG Ala	GAC Asp	1317
AAC Asn	ATA Ile 405	TAC Tyr	ATT Ile	GTC Val	CAC His	TCA Ser	AAC Asn 410	ACG Thr	TGG Trp	TAC Tyr	TCA Ser	ATT Ile 415	GAC Asp	CTA Leu	GAG Glu	1365
AAG Lys 420	GGT Gly	GAG Glu	GCA Ala	AAC Asn	TGC Cys 425	ACT Thr	CGC Arg	CAA Gln	CAC His	ATG Met 430	CAA Gln	GCC Ala	GCA Ala	ATG Met	TAC Tyr	1413
TAC Tyr 435	ATA Ile	CTC Leu	ACC Thr	AGA Arg 440	GGG Gly	TGG Trp	TCA Ser	GAC Asp	AAC Asn 445	GGC Gly	GAC Asp	CCA Pro	ATG Met	TTC Phe	AAT Asn 450	1461
CAA Gln 455	ACA Thr	TGG Trp	GCC Ala	ACC Thr 455	TTT Phe	GCC Ala	ATG Met	AAC Asn 460	ATT Ile	GCC Ala	CCT Pro	GCT Ala	CTA Leu	GTG Val 465	GTG Val	1509
GAC Asp 470	TCA Ser	TCG Ser	TGC Cys 470	CTG Leu	ATA Ile	ATG Met	AAC Asn 475	CTG Leu	CAA Gln	ATT Ile	AAG Lys	ACC Thr 480	TAT Tyr	GGT Gly	CAA Gln	1557
GGC Gly 485	AGC Ser	GGG Gly	AAT Asn	GCA Ala	GCC Ala	ACG Thr	TTC Phe 490	ATC Ile	AAC Asn	AAC Asn	CAC His	CTC Leu 495	TTG Leu	AGC Ser	ACA Thr	1605
CTA Leu 500	GTG Val	CTT Leu	GAC Asp	CAG Gln	TGG Trp	AAC Asn 505	CTG Leu	ATG Met	AGA Arg	CAG Gln	CCC Pro 510	AGA Arg	CCA Pro	GAC Asp	AGC Ser	1653
GAG Glu 515	GAG Glu	TTC Phe	AAA Lys	TCA Ser 520	ATT Ile	GAG Glu	GAC Asp	AAG Lys	CTA Leu	GGT Gly 525	ATC Ile	AAC Asn	TTT Phe	AAG Lys	ATT Ile 530	1701
GAG Glu	AGG Arg	TCC Ser	ATT Ile 535	GAT Asp	GAT Asp	ATC Ile	AGG Arg	GGC Gly	AAG Lys 540	CTG Leu	AGA Arg	CAG Gln	CTT Leu	GTC Val 545	CTC Leu	1749
CTT Leu	GCA Ala	CAA Gln	CCA Pro 550	GGG Gly	TAC Tyr	CTG Leu	AGT Ser	GGG Gly 555	GGG Gly	GTT Val	GAA Glu	CCA Pro	GAA Glu 560	CAA Gln	TCC Ser	1797

AGC Ser	CCA Pro	ACT Thr 565	GTT Val	GAG Glu	CTT Leu	GAC Asp	CTA Leu 570	CTA Leu	GGG Gly	TGG Trp	TCA Ser	GCT Ala 575	ACA Thr	TAC Tyr	AGC Ser	1845
AAA Lys	GAT Asp 580	CTC Leu	GGG Gly	ATC Ile	TAT Tyr	GTG Val 585	CCG Pro	GTG Val	CTT Leu	GAC Asp	AAG Lys 590	GAA Glu	CGC Arg	CTA Leu	TTT Phe	1893
TGT Cys 595	TCT Ser	GCT Ala	GCG Ala	TAT Tyr	CCC Pro 600	AAG Lys	GGA Gly	GTA Val	GAG Glu	AAC Asn 605	AAG Lys	AGT Ser	CTC Leu	AAG Lys	TCC Ser 610	1941
AAA Lys	GTC Val	GGG Gly	ATC Ile	GAG Glu 615	CAG Gln	GCA Ala	TAC Tyr	AAG Lys	GTA Val 620	GTC Val	AGG Arg	TAT Tyr	GAG Glu	GCG Ala 625	TTG Leu	1989
AGG Arg	TTG Leu	GTA Val	GGT Gly 630	GGT Gly	TGG Trp	AAC Asn	TAC Tyr	CCA Pro 635	CTC Leu	CTG Leu	AAC Asn	AAA Lys	GCC Ala 640	TGC Cys	AAG Lys	2037
AAT Asn	AAC Asn	GCA Ala 645	GGC Gly	GCC Ala	GCT Ala	CGG Arg	CGG Arg 650	CAT His	CTG Leu	GAG Glu	GCC Ala 655	AAG Lys	GGG Gly	TTC Phe	CCA Pro	2085
CTC Leu	GAC Asp 660	GAG Glu	TTC Phe	CTA Leu	GCC Ala	GAG Glu 665	TGG Trp	TCT Ser	GAG Glu	CTG Leu	TCA Ser 670	GAG Glu	TTC Phe	GGT Gly	GAG Glu	2133
GCC Ala 675	TTC Phe	GAA Glu	GGC Gly	TTC Phe	AAT Asn 680	ATC Ile	AAG Lys	CTG Leu	ACC Thr	GTA Val 685	ACA Thr	TCT Ser	GAG Glu	AGC Ser	CTA Leu 690	2181
GCC Ala	GAA Glu	CTG Leu	AAC Asn	AAG Lys 695	CCA Pro	GTA Val	CCC Pro	CCC Pro	AAG Lys 700	CCC Pro	CCA Pro	AAT Asn	GTC Val	AAC Asn	AGA Arg 705	2229
CCA Pro	GTC Val	AAC Asn	ACT Thr 710	GGG Gly	GGA Gly	CTC Leu	AAG Lys	GCA Ala 715	GTC Val	AGC Ser	AAC Asn	GCC Ala	CTC Leu 720	AAG Lys	ACC Thr	2277
GGT Gly	CGG Arg	TAC Tyr 725	AGG Arg	AAC Asn	GAA Glu	GCC Ala	GGA Gly 730	CTG Leu	AGT Ser	GGT Gly	CTC Leu	GTC Val 735	CTT Leu	CTA Leu	GCC Ala	2325
ACA Thr	GCA Ala 740	AGA Arg	AGC Ser	CGT Arg	CTG Leu	CAA Gln 745	GAT Asp	GCA Ala	GTT Val	AAG Lys	GCC Ala 750	AAG Lys	GCA Ala	GAA Glu	GCC Ala	2373
GAG Glu 755	AAA Lys	CTC Leu	CAC His	AAG Lys	TCC Ser 760	AAG Lys	CCA Pro	GAC Asp	GAC Asp	CCC Pro 765	GAT Asp	GCA Ala	GAC Asp	TGG Trp	TTC Phe 770	2421

GAA AGA TCA GAA ACT CTG TCA GAC CTT CTG GAG AAA GCC GAC ATC GCC	2469
Glu Arg Ser Glu Thr Leu Ser Asp Leu Leu Glu Lys Ala Asp Ile Ala	
775 780 785	
AGC AAG GTC GCC CAC TCA GCA CTC GTG GAA ACA AGC GAC GCC CTT GAA	2517
Ser Lys Val Ala His Ser Ala Leu Val Glu Thr Ser Asp Ala Leu Glu	
790 795 800	
GCA GTT CAG TCG ACT TCC GTG TAC ACC CCC AAG TAC CCA GAA GTC AAG	2565
Ala Val Gln Ser Thr Ser Val Tyr Thr Pro Lys Tyr Pro Glu Val Lys	
805 810 815	
AAC CCA CAG ACC GCC TCC AAC CCC GTT GTT GGG CTC CAC CTG CCC GCC	2613
Asn Pro Gln Thr Ala Ser Asn Pro Val Val Gly Leu His Leu Pro Ala	
820 825 830	
AAG AGA GCC ACC GGT GTC CAG GCC GCT CTT CTC GGA GCA GGA ACG AGC	2661
Lys Arg Ala Thr Gly Val Gln Ala Ala Leu Leu Gly Ala Gly Thr Ser	
835 840 845 850	
AGA CCA ATG GGG ATG GAG GCC CCA ACA CGG TCC AAG AAC GCC GTG AAA	2709
Arg Pro Met Gly Met Glu Ala Pro Thr Arg Ser Lys Asn Ala Val Lys	
855 860 865	
ATG GCC AAA CGG CGG CAA CGC CAA AAG GAG AGC CGC TAACAGCCAT	2755
Met Ala Lys Arg Arg Gln Arg Gln Lys Glu Ser Arg	
870 875	
GATGGGAACC ACTCAAGAAG AGGACACTAA TCCCAGACCC CGTATCCCCG GCCTTCGCCT	2815
SCGGGGGGCCCC CC	2827

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 878 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Ser	Asp	Ile	Phe	Asn	Ser	Pro	Gln	Ala	Arg	Ser	Thr	Ile	Ser	Ala
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Ala	Phe	Gly	Ile	Lys	Pro	Thr	Ala	Gly	Gln	Asp	Val	Glu	Glu	Leu	Leu
		20						25					30		
Ile	Pro	Lys	Val	Trp	Val	Pro	Pro	Glu	Asp	Pro	Leu	Ala	Ser	Pro	Ser
		35					40					45			

Arg	Leu	Ala	Lys	Phe	Leu	Arg	Glu	Asn	Gly	Tyr	Lys	Val	Leu	Gln	Pro	50	55	60
Arg	Ser	Leu	Pro	Glu	Asn	Glu	Glu	Tyr	Glu	Thr	Asp	Gln	Ile	Leu	Pro	65	70	75
Asp	Leu	Ala	Trp	Met	Arg	Gln	Ile	Glu	Gly	Ala	Val	Leu	Lys	Pro	Thr	85	90	95
Leu	Ser	Leu	Pro	Ile	Gly	Asp	Gln	Glu	Tyr	Phe	Pro	Lys	Tyr	Tyr	Pro	100	105	110
Thr	His	Arg	Pro	Ser	Lys	Glu	Lys	Pro	Asn	Ala	Tyr	Pro	Pro	Asp	Ile	115	120	125
Ala	Leu	Leu	Lys	Gln	Met	Ile	Tyr	Leu	Phe	Leu	Gln	Val	Pro	Glu	Ala	130	135	140
Asn	Glu	Gly	Leu	Lys	Asp	Glu	Val	Thr	Leu	Leu	Thr	Gln	Asn	Ile	Arg	145	150	155
Asp	Lys	Ala	Tyr	Gly	Ser	Gly	Thr	Tyr	Met	Gly	Gln	Ala	Asn	Arg	Leu	165	170	175
Val	Ala	Met	Lys	Glu	Val	Ala	Thr	Gly	Arg	Asn	Pro	Asn	Lys	Asp	Pro	180	185	190
Leu	Lys	Leu	Gly	Tyr	Thr	Phe	Glu	Ser	Ile	Ala	Gln	Leu	Leu	Asp	Ile	195	200	205
Thr	Leu	Pro	Val	Gly	Pro	Pro	Gly	Glu	Asp	Asp	Lys	Pro	Trp	Val	Pro	210	215	220
Leu	Thr	Arg	Val	Pro	Ser	Arg	Met	Leu	Val	Leu	Thr	Gly	Asp	Val	Asp	225	230	235
Gly	Asp	Phe	Glu	Val	Glu	Asp	Tyr	Leu	Pro	Lys	Ile	Asn	Leu	Lys	Ser	245	250	255
Ser	Ser	Gly	Leu	Pro	Tyr	Val	Gly	Arg	Thr	Lys	Gly	Glu	Thr	Ile	Gly	260	265	270
Glu	Met	Ile	Ala	Ile	Ser	Asn	Gln	Phe	Leu	Arg	Glu	Leu	Ser	Thr	Leu	275	280	285
Leu	Lys	Gln	Gly	Ala	Gly	Thr	Lys	Gly	Ser	Asn	Lys	Lys	Lys	Leu	Leu	290	295	300
Ser	Met	Leu	Ser	Asp	Tyr	Trp	Tyr	Leu	Ser	Cys	Gly	Leu	Leu	Phe	Pro	305	310	315
																		320

Lys	Ala	Glu	Arg	Tyr	Asp	Lys	Ser	Thr	Trp	Leu	Thr	Lys	Thr	Arg	Asn	
				325					330					335		
Ile	Trp	Ser	Ala	Pro	Ser	Pro	Thr	His	Leu	Met	Ile	Ser	Met	Ile	Thr	
			340					345					350			
Trp	Pro	Val	Met	Ser	Asn	Ser	Pro	Asn	Asn	Val	Leu	Asn	Ile	Glu	Gly	
		355					360					365				
Cys	Pro	Ser	Leu	Tyr	Lys	Phe	Asn	Pro	Phe	Arg	Gly	Gly	Leu	Asn	Arg	
	370					375					380					
Ile	Val	Glu	Trp	Ile	Leu	Ala	Pro	Glu	Glu	Pro	Lys	Ala	Leu	Val	Tyr	
385					390					395					400	
Ala	Asp	Asn	Ile	Tyr	Ile	Val	His	Ser	Asn	Thr	Trp	Tyr	Ser	Ile	Asp	
			405						410					415		
Leu	Glu	Lys	Gly	Glu	Ala	Asn	Cys	Thr	Arg	Gln	His	Met	Gln	Ala	Ala	
			420					425					430			
Met	Tyr	Tyr	Ile	Leu	Thr	Arg	Gly	Trp	Ser	Asp	Asn	Gly	Asp	Pro	Met	
		435					440					445				
Phe	Asn	Gln	Thr	Trp	Ala	Thr	Phe	Ala	Met	Asn	Ile	Ala	Pro	Ala	Leu	
	450					455					460					
Val	Val	Asp	Ser	Ser	Cys	Leu	Ile	Met	Asn	Leu	Gln	Ile	Lys	Thr	Tyr	
465					470					475					480	
Gly	Gln	Gly	Ser	Gly	Asn	Ala	Ala	Thr	Phe	Ile	Asn	Asn	His	Leu	Leu	
				485					490					495		
Ser	Thr	Leu	Val	Leu	Asp	Gln	Trp	Asn	Leu	Met	Arg	Gln	Pro	Arg	Pro	
			500					505					510			
Asp	Ser	Glu	Glu	Phe	Lys	Ser	Ile	Glu	Asp	Lys	Leu	Gly	Ile	Asn	Phe	
		515					520					525				
Lys	Ile	Glu	Arg	Ser	Ile	Asp	Asp	Ile	Arg	Gly	Lys	Leu	Arg	Gln	Leu	
	530					535					540					
Val	Leu	Leu	Ala	Gln	Pro	Gly	Tyr	Leu	Ser	Gly	Gly	Val	Glu	Pro	Glu	
545					550					555					560	
Gln	Ser	Ser	Pro	Thr	Val	Glu	Leu	Asp	Leu	Leu	Gly	Trp	Ser	Ala	Thr	
				565					570					575		
Tyr	Ser	Lys	Asp	Leu	Gly	Ile	Tyr	Val	Pro	Val	Leu	Asp	Lys	Glu	Arg	
			580					585					590			

Leu	Phe	Cys	Ser	Ala	Ala	Tyr	Pro	Lys	Gly	Val	Glu	Asn	Lys	Ser	Leu
		595					600					605			
Lys	Ser	Lys	Val	Gly	Ile	Glu	Gln	Ala	Tyr	Lys	Val	Val	Arg	Tyr	Glu
	610					615					620				
Ala	Leu	Arg	Leu	Val	Gly	Gly	Trp	Asn	Tyr	Pro	Leu	Leu	Asn	Lys	Ala
625					630					635					640
Cys	Lys	Asn	Asn	Ala	Gly	Ala	Ala	Arg	Arg	His	Leu	Glu	Ala	Lys	Gly
				645					650					655	
Phe	Pro	Leu	Asp	Glu	Phe	Leu	Ala	Glu	Trp	Ser	Glu	Leu	Ser	Glu	Phe
			660					665					670		
Gly	Glu	Ala	Phe	Glu	Gly	Phe	Asn	Ile	Lys	Leu	Thr	Val	Thr	Ser	Glu
		675					680					685			
Ser	Leu	Ala	Glu	Leu	Asn	Lys	Pro	Val	Pro	Pro	Lys	Pro	Pro	Asn	Val
	690					695					700				
Asn	Arg	Pro	Val	Asn	Thr	Gly	Gly	Leu	Lys	Ala	Val	Ser	Asn	Ala	Leu
705					710					715					720
Lys	Thr	Gly	Arg	Tyr	Arg	Asn	Glu	Ala	Gly	Leu	Ser	Gly	Leu	Val	Leu
				725					730					735	
Leu	Ala	Thr	Ala	Arg	Ser	Arg	Leu	Gln	Asp	Ala	Val	Lys	Ala	Lys	Ala
			740					745					750		
Glu	Ala	Glu	Lys	Leu	His	Lys	Ser	Lys	Pro	Asp	Asp	Pro	Asp	Ala	Asp
		755					760					765			
Trp	Phe	Glu	Arg	Ser	Glu	Thr	Leu	Ser	Asp	Leu	Leu	Glu	Lys	Ala	Asp
	770					775					780				
Ile	Ala	Ser	Lys	Val	Ala	His	Ser	Ala	Leu	Val	Glu	Thr	Ser	Asp	Ala
785					790					795					800
Leu	Glu	Ala	Val	Gln	Ser	Thr	Ser	Val	Tyr	Thr	Pro	Lys	Tyr	Pro	Glu
				805					810					815	
Val	Lys	Asn	Pro	Gln	Thr	Ala	Ser	Asn	Pro	Val	Val	Gly	Leu	His	Leu
			820					825					830		
Pro	Ala	Lys	Arg	Ala	Thr	Gly	Val	Gln	Ala	Ala	Leu	Leu	Gly	Ala	Gly
		835					840					845			
Thr	Ser	Arg	Pro	Met	Gly	Met	Glu	Ala	Pro	Thr	Arg	Ser	Lys	Asn	Ala
	850					855					860				

Val Lys Met Ala Lys Arg Arg Gln Arg Gln Lys Glu Ser Arg
 865 870 875

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3261 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 97..531

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGATACGATC GGTCTGACCC CGGGGGAGTC ACCCGGGGAC AGGCCGTCAA GGCCTTGTTT	60
CAGGATGGGA CTCCTCCTTC TACAACGCTA TCATTG ATG GTT AGT AGA GAT CAG	114
Met Val Ser Arg Asp Gln	
1 5	
ACA AAC GAT CGC AGC GAT GAC AAA CCT GCA AGA TCA AAC CCA ACA GAT	162
Thr Asn Asp Arg Ser Asp Asp Lys Pro Ala Arg Ser Asn Pro Thr Asp	
10 15 20	
TGT TCC GTT CAT ACG GAG CCT TCT GAT GCC AAC AAC CGG ACC GGC GTC	210
Cys Ser Val His Thr Glu Pro Ser Asp Ala Asn Asn Arg Thr Gly Val	
25 30 35	
CAT TCC GGA CGA CAC CCT GGA GAA GCA CAC TCT CAG GTC AGA GAC CTC	258
His Ser Gly Arg His Pro Gly Glu Ala His Ser Gln Val Arg Asp Leu	
40 45 50	
GAC CTA CAA TTT GAC TGT GGG GGA CAC AGG GTC AGG GCT AAT TGT CTT	306
Asp Leu Gln Phe Asp Cys Gly Gly His Arg Val Arg Ala Asn Cys Leu	
55 60 65 70	
TTT CCC TGG ATT CCC TGG CTC AAT TGT GGG TGC TCA CTA CAC ACT GCA	354
Phe Pro Trp Ile Pro Trp Leu Asn Cys Gly Cys Ser Leu His Thr Ala	
75 80 85	

GGG CAA TGG GAA CTA CAA GTT CGA TCA GAT GCT CCT GAC TGC CCA GAA	402
Gly Gln Trp Glu Leu Gln Val Arg Ser Asp Ala Pro Asp Cys Pro Glu	
90 95 100	
CCT ACC GGC CAG TTA CAA CTA CTG CAG GCT AGT GAG TCG GAG TCT CAC	450
Pro Thr Gly Gln Leu Gln Leu Leu Gln Ala Ser Glu Ser Glu Ser His	
105 110 115	
AGT GAG GTC AAG CAC ACT TCC TGG TGG CGT TTA TGC ACT AAA CGG CAC	498
Ser Glu Val Lys His Thr Ser Trp Trp Arg Leu Cys Thr Lys Arg His	
120 125 130	
CAT AAA CGC CGT GAC CTT CCA AGG AAG CCT GAG TGA ACTGACA GATGTTAGCT	551
His Lys Arg Arg Asp Leu Pro Arg Lys Pro Glu	
135 140 145	
ACAATGGGTT GATGTCTGCA ACAGCCAACA TCAACGACAA AATTGGGAAC GTCCTAGTAG	611
GGGAAGGGGT CACCGTCCTC AGCTTACCCA CATCATATGA TCTTGGGTAT GTGAGGCTTG	671
GTGACCCCAT TCCCGCAATA GGGCTTGACC CAAAAATGGT AGCCACATGT GACAGCAGTG	731
ACAGGCCCAG AGTCTACACC ATA ACTGCAG CCGATGATTA CCAATTCTCA TCACAGTACC	791
AACCAGGTGG GGTAACAATC ACACTGTTCT CAGCCAACAT TGATGCCATC ACAAGCCTCA	851
GCGTTGGGGG AGAGCTCGTG TTTCAAACAA GCGTCCACGG CCTTGTTACTG GGCGCCACCA	911
TCTACCTCAT AGGCTTTGAT GGGACAACGG TAATCACCAG GGCTGTGGCC GCAAACAATG	971
GGCTGACGAC CGGCACCGAC AACCTTATGC CATTCAATCT TGTGATTCCA ACAAACGAGA	1031
TAAACCAGCC AATCACATCC ATCAA ACTGG AGATAGTGAC CTCCAAAAGT GGTGGTCAGG	1091
CAGGGGATCA GATGTCATGG TCGGCAAGAG GGAGCCTAGC AGTGACGATC CATGGTGGCA	1151
ACTATCCAGG GGCCCTCCGT CCCGTCACGC TAGTGGCCTA CGAAAGAGTG GCAACAGGAT	1211
CCGTCGTTAC GGTCGCTGGG GTGAGCAACT TCGAGCTGAT CCCAAATCCT GAACTAGCAA	1271
AGAACCTGGT TACAGAATAC GGCCGATTTG ACCCAGGAGC CATGAACTAC ACAA AATTGA	1331
TACTGAGTGA GAGGGACCGT CTTGGCATCA AGACCGTCTG GCCAACAAGG GAGTACACTG	1391
ACTTTCGTGA ATACTTCATG GAGGTGGCCG ACCTCAACTC TCCCCTGAAG ATTGCAGGAG	1451
CATTCGGCTT CAAAGACATA ATCCGGGCCA TAAGGAGGAT AGCTGTGCCG GTGGTCTCCA	1511
CATTGTTCCC ACCTGCCGCT CCCCTAGCCC ATGCAATTGG GGAAGGTGTA GACTACCTGC	1571
TGGGCGATGA GGCACAGGCT GCTTCAGGAA CTGCTCGAGC CGCGTCAGGA AAAGCAAGAG	1631

CTGCCTCAGG	CCGCATAAGG	CAGCTGACTC	TCGCCGCCGA	CAAGGGGTAC	GAGGTAGTCG	1691
CGAATCTATT	CCAGGTGCCC	CAGAATCCCG	TAGTCGACGG	GATTCTTGCT	TCACCTGGGG	1751
TACTCCGCGG	TGCACACAAC	CTCGACTGCG	TGTTAAGAGA	GGGTGCCACG	CTATTC CCTG	1811
TGGTTATTAC	GACAGTGGAA	GACGCCATGA	CACCCAAAGC	ATTGAACAGC	AAAATGTTTG	1871
CTGTCATTGA	AGGCGTGCGA	GAAGACCTCC	AACCTCCATC	TCAAAGAGGA	TCCTTCATAC	1931
GAACCTCTCTC	TGGACACAGA	GTCTATGGAT	ATGCTCCAGA	TGGGGTACTT	CCACTGGAGA	1991
CTGGGAGAGA	CTACACCGTT	GTCCCAATAG	ATGATGTCTG	GGACGACAGC	ATTATGCTGT	2051
CCAAAGATCC	CATACCTCCT	ATTGTGGGAA	ACAGTGGAAA	TCTAGCCATA	GCTTACATGG	2111
ATGTGTTTCG	ACCCAAAGTC	CCAATCCATG	TGGCTATGAC	GGGAGCCCTC	AATGCTTG TG	2171
GCGAGATTGA	GAAAGTAAGC	TTTAGAAGCA	CCAAGCTCGC	CACTGCACAC	CGACTTG GCC	2231
TTAGGTTGGC	TGGTCCCGGA	GCATTCGATG	TAAACACCGG	GCCCAACTGG	GCAACGTTCA	2291
TCAAACGTTT	CCCTCACAAT	CCACGCGACT	GGGACAGGCT	CCCCTACCTC	AACCTACCAT	2351
ACCTTCCACC	CAATGCAGGA	CGCCAGTACC	ACCTTGCCAT	GGCTGCATCA	GAGTTCAAAG	2411
AGACCCCCGA	ACTCGAGAGT	GCCGTCAGAG	CAATGGAAGC	AGCAGCCAAC	GTGGACCCAC	2471
TATTCCAATC	TGCACTCAGT	GTGTTCATGT	GGCTGGAAGA	GAATGGGATT	GTGACTGACA	2531
TGGCCAACTT	CGCACTCAGC	GACCCGAACG	CCCATCGGAT	GCGAAATTTT	CTTGCAAACG	2591
CACCACAAGC	AGGCAGCAAG	TCGCAAAGGG	CCAAGTACGG	GACAGCAGGC	TACGGAGTGG	2651
AGGCTCGGGG	CCCCACACCA	GAGGAAGCAC	AGAGGGAAAA	AGACACACGG	ATCTCAAAGA	2711
AGATGGAGAC	CATGGGCATC	TACTTTGCAA	CACCAGAATG	GGTAGCACTC	AATGGGCACC	2771
GAGGGCCAAG	CCCCGGCCAG	CTAAAGTACT	GGCAGAACAC	ACGAGAAATA	CCGGACCCAA	2831
ACGAGGACTA	TCTAGACTAC	GTGCATGCAG	AGAAGAGCCG	GTTGGCATCA	GAAGAACAAA	2891
TCCTAAGGGC	AGCTACGTCG	ATCTACGGGG	CTCCAGGACA	GGCAGAGCCA	CCCCAAGCTT	2951
TCATAGACGA	AGTTGCCAAA	GTCTATGAAA	TCAACCATGG	ACGTGGCCCA	AACCAAGAAC	3011
AGATGAAAGA	TCTGCTCTTG	ACTGCGATGG	AGATGAAGCA	TCGCAATCCC	AGGCGGGCTC	3071
TACCAAAGCC	CAAGCCAAAA	CCCAATGCTC	CAACACAGAG	ACCCCCTGGT	CGGCTGGGCC	3131
GCTGGATCAG	GACCGTCTCT	GATGAGGACC	TTGAGTGAGG	CTCCTGGGAG	TCTCCCGACA	3191

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

[illegible]

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3261 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:131..3166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGATACGATC GGTCTGACCC CGGGGGAGTC ACCCGGGGAC AGGCCGTCAA GGCCTTG TTC	60
GAGGATG GGA CTCCTCCTTC TACAACGCTA TCATTGATGG TTAGTAGAGA TCAGACAAAC	120
GATCGCAGCG ATG ACA AAC CTG CAA GAT CAA ACC CAA CAG ATT GTT CCG	169
Met Thr Asn Leu Gln Asp Gln Thr Gln Gln Ile Val Pro	
1 5 10	
TTC ATA CGG AGC CTT CTG ATG CCA ACA ACC GGA CCG GCG TCC ATT CCG	217
Phe Ile Arg Ser Leu Leu Met Pro Thr Thr Gly Pro Ala Ser Ile Pro	
15 20 25	
GAC GAC ACC CTG GAG AAG CAC ACT CTC AGG TCA GAG ACC TCG ACC TAC	265
Asp Asp Thr Leu Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr	
30 35 40 45	
AAT TTG ACT GTG GGG GAC ACA GGG TCA GGG CTA ATT GTC TTT TTC CCT	313
Asn Leu Thr Val Gly Asp Thr Gly Ser Gly Leu Ile Val Phe Phe Pro	
50 55 60	
GGA TTC CCT GGC TCA ATT GTG GGT GCT CAC TAC ACA CTG CAG GGC AAT	361
Gly Phe Pro Gly Ser Ile Val Gly Ala His Tyr Thr Leu Gln Gly Asn	
65 70 75	
GGG AAC TAC AAG TTC GAT CAG ATG CTC CTG ACT GCC CAG AAC CTA CCG	409
Gly Asn Tyr Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro	
80 85 90	
GCC AGT TAC AAC TAC TGC AGG CTA GTG AGT CGG AGT CTC ACA GTG AGG	457
Ala Ser Tyr Asn Tyr Cys Arg Leu Val Ser Arg Ser Leu Thr Val Arg	
95 100 105	

TCA Ser 110	AGC Ser	ACA Thr	CTT Leu	CCT Pro	GGT Gly 115	GGC Gly	GTT Val	TAT Tyr	GCA Ala	CTA Leu 120	AAC Asn	GGC Gly	ACC Thr	ATA Ile	AAC Asn 125	505
GCC Ala	GTG Val	ACC Thr	TTC Phe	CAA Gln 130	GGA Gly	AGC Ser	CTG Leu	AGT Ser	GAA Glu 135	CTG Leu	ACA Thr	GAT Asp	GTT Val	AGC Ser 140	TAC Tyr	553
AAT Asn	GGG Gly	TTG Leu	ATG Met 145	TCT Ser	GCA Ala	ACA Thr	GCC Ala	AAC Asn 150	ATC Ile	AAC Asn	GAC Asp	AAA Lys	ATT Ile 155	GGG Gly	AAC Asn	601
GTC Val	CTA Leu	GTA Val 160	GGG Gly	GAA Glu	GGG Gly	GTC Val	ACC Thr 165	GTC Val	CTC Leu	AGC Ser	TTA Leu	CCC Pro 170	ACA Thr	TCA Ser	TAT Tyr	649
GAT Asp	CTT Leu 175	GGG Gly	TAT Tyr	GTG Val	AGG Arg	CTT Leu 180	GGT Gly	GAC Asp	CCC Pro	ATT Ile 185	CCC Pro	GCA Ala	ATA Ile	GGG Gly	CTT Leu	697
GAC Asp 190	CCA Pro	AAA Lys	ATG Met	GTA Val	GCC Ala 195	ACA Thr	TGT Cys	GAC Asp	AGC Ser	AGT Ser 200	GAC Asp	AGG Arg	CCC Pro	AGA Arg	GTC Val 205	745
TAC Tyr	ACC Thr	ATA Ile	ACT Thr	GCA Ala 210	GCC Ala	GAT Asp	GAT Asp	TAC Tyr	CAA Gln 215	TTC Phe	TCA Ser	TCA Ser	CAG Gln	TAC Tyr 220	CAA Gln	793
GCA Pro	GGT Gly	GGG Gly	GTA Val 225	ACA Thr	ATC Ile	ACA Thr	CTG Leu	TTC Phe 230	TCA Ser	GCC Ala	AAC Asn	ATT Ile 235	GAT Asp	GCC Ala	ATC Ile	841
ACA Thr	AGC Ser	CTC Leu 240	AGC Ser	GTT Val	GGG Gly	GGA Gly	GAG Glu 245	CTC Leu	GTG Val	TTT Phe	CAA Gln	ACA Thr 250	AGC Ser	GTC Val	CAC His	889
GGC Gly 255	CTT Leu	GTA Val	CTG Leu	GGC Gly	GCC Ala 260	ACC Thr	ATC Ile	TAC Tyr	CTC Leu	ATA Ile	GGC Gly 265	TTT Phe	GAT Asp	GGG Gly	ACA Thr	937
ACG Thr 270	GTA Val	ATC Ile	ACC Thr	AGG Arg	GCT Ala 275	GTG Val	GCC Ala	GCA Ala	AAC Asn	AAT Asn 280	GGG Gly	CTG Leu	ACG Thr	ACC Thr	GGC Gly 285	985
ACC Thr	GAC Asp	AAC Asn	CTT Leu	ATG Met 290	CCA Pro	TTC Phe	AAT Asn	CTT Leu 295	GTG Val	ATT Ile	CCA Pro	ACA Thr	AAC Asn	GAG Glu 300	ATA Ile	1033
ACC Thr	CAG Gln	CCA Pro	ATC Ile 305	ACA Thr	TCC Ser	ATC Ile	AAA Lys	CTG Leu 310	GAG Glu	ATA Ile	GTG Val	ACC Thr	TCC Ser 315	AAA Lys	AGT Ser	1081

GGT Gly	GGT Gly	CAG Gln 320	GCA Ala	GGG Gly	GAT Asp	CAG Gln 325	ATG Met	TCA Ser	TGG Trp	TCG Ser	GCA Ala 330	AGA Arg	GGG Gly	AGC Ser	CTA Leu	1129
GCA Ala 335	GTG Val	ACG Thr	ATC Ile	CAT His	GGT Gly 340	GGC Gly	AAC Asn	TAT Tyr	CCA Pro	GGG Gly 345	GCC Ala	CTC Leu	CGT Arg	CCC Pro	GTC Val	1177
ACG Thr 350	CTA Leu	GTG Val	GCC Ala	TAC Tyr	GAA Glu 355	AGA Arg	GTG Val	GCA Ala	ACA Thr	GGA Gly 360	TCC Ser	GTC Val	GTT Val	ACG Thr	GTC Val 365	1225
GCT Ala	GGG Gly	GTG Val	AGC Ser	AAC Asn 370	TTC Phe	GAG Glu	CTG Leu	ATC Ile	CCA Pro 375	AAT Asn	CCT Pro	GAA Glu	CTA Leu	GCA Ala 380	AAG Lys	1273
AAC Asn	CTG Leu	GTT Val	ACA Thr 385	GAA Glu	TAC Tyr	GGC Gly	CGA Arg	TTT Phe 390	GAC Asp	CCA Pro	GGA Gly	GCC Ala	ATG Met 395	AAC Asn	TAC Tyr	1321
ACA Thr	AAA Lys	TTG Leu 400	ATA Ile	CTG Leu	AGT Ser	GAG Glu 405	AGG Arg	GAC Asp	CGT Arg	CTT Leu	GGC Gly 410	ATC Ile	AAG Lys	ACC Thr	GTC Val	1369
TGG Trp 415	CCA Pro	ACA Thr	AGG Arg	GAG Glu	TAC Tyr	ACT Thr 420	GAC Asp	TTT Phe	CGT Arg	GAA Glu 425	TAC Tyr	TTC Phe	ATG Met	GAG Glu	GTG Val	1417
GCC Ala 430	GAC Asp	CTC Leu	AAC Asn	TCT Ser	CCC Pro 435	CTG Leu	AAG Lys	ATT Ile	GCA Ala	GGA Gly 440	GCA Ala	TTC Phe	GGC Gly	TTC Phe	AAA Lys 445	1465
GAC Asp	ATA Ile	ATC Ile	CGG Arg	GCC Ala 450	ATA Ile	AGG Arg	AGG Arg	ATA Ile	GCT Ala 455	GTG Val	CCG Pro	GTG Val	GTC Val	TCC Ser	ACA Thr 460	1513
TTG Leu	TTC Phe	CCA Pro	CCT Pro 465	GCC Ala	GCT Ala	CCC Pro	CTA Leu	GCC Ala 470	CAT His	GCA Ala	ATT Ile	GGG Gly 475	GAA Glu	GGT Gly	GTA Val	1561
GAC Asp	TAC Tyr	CTG Leu 480	CTG Leu	GGC Gly	GAT Asp	GAG Glu	GCA Ala 485	CAG Gln	GCT Ala	GCT Ala	TCA Ser	GGA Gly 490	ACT Thr	GCT Ala	CGA Arg	1609
GCC Ala 495	GCG Ala	TCA Ser	GGA Gly	AAA Lys	GCA Ala	AGA Arg 500	GCT Ala	GCC Ala	TCA Ser	GGC Gly 505	CGC Arg	ATA Ile	AGG Arg	CAG Gln	CTG Leu	1657
ACT Thr 510	CTC Leu	GCC Ala	GCC Ala	GAC Asp	AAG Lys 515	GGG Gly	TAC Tyr	GAG Glu	GTA Val	GTC Val 520	GCG Ala	AAT Asn	CTA Leu	TTC Phe	CAG Gln 525	1705

GTG	CCC	CAG	AAT	CCC	GTA	GTC	GAC	GGG	ATT	CTT	GCT	TCA	CCT	GGG	GTA	1753
Val	Pro	Gln	Asn	Pro	Val	Val	Asp	Gly	Ile	Leu	Ala	Ser	Pro	Gly	Val	
				530					535					540		
CTC	CGC	GGT	GCA	CAC	AAC	CTC	GAC	TGC	GTG	TTA	AGA	GAG	GGT	GCC	ACG	1801
Leu	Arg	Gly	Ala	His	Asn	Leu	Asp	Cys	Val	Leu	Arg	Glu	Gly	Ala	Thr	
			545					550					555			
CTA	TTC	CCT	GTG	GTT	ATT	ACG	ACA	GTG	GAA	GAC	GCC	ATG	ACA	CCC	AAA	1849
Leu	Phe	Pro	Val	Val	Ile	Thr	Thr	Val	Glu	Asp	Ala	Met	Thr	Pro	Lys	
		560					565					570				
GCA	TTG	AAC	AGC	AAA	ATG	TTT	GCT	GTC	ATT	GAA	GGC	GTG	CGA	GAA	GAC	1897
Ala	Leu	Asn	Ser	Lys	Met	Phe	Ala	Val	Ile	Glu	Gly	Val	Arg	Glu	Asp	
	575					580					585					
CTC	CAA	CCT	CCA	TCT	CAA	AGA	GGA	TCC	TTC	ATA	CGA	ACT	CTC	TCT	GGA	1945
Leu	Gln	Pro	Pro	Ser	Gln	Arg	Gly	Ser	Phe	Ile	Arg	Thr	Leu	Ser	Gly	
590					595					600					605	
CAC	AGA	GTC	TAT	GGA	TAT	GCT	CCA	GAT	GGG	GTA	CTT	CCA	CTG	GAG	ACT	1993
His	Arg	Val	Tyr	Gly	Tyr	Ala	Pro	Asp	Gly	Val	Leu	Pro	Leu	Glu	Thr	
				610					615					620		
GGG	AGA	GAC	TAC	ACC	GTT	GTC	CCA	ATA	GAT	GAT	GTC	TGG	GAC	GAC	AGC	2041
Gly	Arg	Asp	Tyr	Thr	Val	Val	Pro	Ile	Asp	Asp	Val	Trp	Asp	Asp	Ser	
			625				630						635			
ATT	ATG	CTG	TCC	AAA	GAT	CCC	ATA	CCT	CCT	ATT	GTG	GGA	AAC	AGT	GGA	2089
Ile	Met	Leu	Ser	Lys	Asp	Pro	Ile	Pro	Pro	Ile	Val	Gly	Asn	Ser	Gly	
		640					645					650				
AAT	CTA	GCC	ATA	GCT	TAC	ATG	GAT	GTG	TTT	CGA	CCC	AAA	GTC	CCA	ATC	2137
Asn	Leu	Ala	Ile	Ala	Tyr	Met	Asp	Val	Phe	Arg	Pro	Lys	Val	Pro	Ile	
	655					660					665					
CAT	GTG	GCT	ATG	ACG	GGA	GCC	CTC	AAT	GCT	TGT	GGC	GAG	ATT	GAG	AAA	2185
His	Val	Ala	Met	Thr	Gly	Ala	Leu	Asn	Ala	Cys	Gly	Glu	Ile	Glu	Lys	
670					675					680					685	
GTA	AGC	TTT	AGA	AGC	ACC	AAG	CTC	GCC	ACT	GCA	CAC	CGA	CTT	GGC	CTT	2233
Val	Ser	Phe	Arg	Ser	Thr	Lys	Leu	Ala	Thr	Ala	His	Arg	Leu	Gly	Leu	
				690					695					700		
AGG	TTG	GCT	GGT	CCC	GGA	GCA	TTC	GAT	GTA	AAC	ACC	GGG	CCC	AAC	TGG	2281
Arg	Leu	Ala	Gly	Pro	Gly	Ala	Phe	Asp	Val	Asn	Thr	Gly	Pro	Asn	Trp	
			705					710					715			
GCA	ACG	TTC	ATC	AAA	CGT	TTC	CCT	CAC	AAT	CCA	CGC	GAC	TGG	GAC	AGG	2329
Ala	Thr	Phe	Ile	Lys	Arg	Phe	Pro	His	Asn	Pro	Arg	Asp	Trp	Asp	Arg	
		720					725						730			

CTC Leu	CCC Pro	TAC Tyr	CTC Leu	AAC Asn	CTA Leu	CCA Pro	TAC Tyr	CTT Leu	CCA Pro	CCC Pro	AAT Asn	GCA Ala	GGA Gly	CGC Arg	CAG Gln	2377
	735					740					745					
TAC Tyr	CAC His	CTT Leu	GCC Ala	ATG Met	GCT Ala	GCA Ala	TCA Ser	GAG Glu	TTC Phe	AAA Lys	GAG Glu	ACC Thr	CCC Pro	GAA Glu	CTC Leu	2425
	750				755					760					765	
GAG Glu	AGT Ser	GCC Ala	GTC Val	AGA Arg	GCA Ala	ATG Met	GAA Glu	GCA Ala	GCA Ala	GCC Ala	AAC Asn	GTG Val	GAC Asp	CCA Pro	CTA Leu	2473
				770						775					780	
TTC Phe	CAA Gln	TCT Ser	GCA Ala	CTC Leu	AGT Ser	GTG Val	TTC Phe	ATG Met	TGG Trp	CTG Leu	GAA Glu	GAG Glu	AAT Asn	GGG Gly	ATT Ile	2521
			785					790						795		
GTG Val	ACT Thr	GAC Asp	ATG Met	GCC Ala	AAC Asn	TTC Phe	GCA Ala	CTC Leu	AGC Ser	GAC Asp	CCG Pro	AAC Asn	GCC Ala	CAT His	CGG Arg	2569
		800					805						810			
ATG Met	CGA Arg	AAT Asn	TTT Phe	CTT Leu	GCA Ala	AAC Asn	GCA Ala	CCA Pro	CAA Gln	GCA Ala	GGC Gly	AGC Ser	AAG Lys	TCG Ser	CAA Gln	2617
	815					820					825					
AGG Arg	GCC Ala	AAG Lys	TAC Tyr	GGG Gly	ACA Thr	GCA Ala	GGC Gly	TAC Tyr	GGA Gly	GTG Val	GAG Glu	GCT Ala	CGG Arg	GGC Gly	CCC Pro	2665
	830				835					840					845	
ACA Thr	CCA Pro	GAG Glu	GAA Glu	GCA Ala	CAG Gln	AGG Arg	GAA Glu	AAA Lys	GAC Asp	ACA Thr	CGG Arg	ATC Ile	TCA Ser	AAG Lys	AAG Lys	2713
				850					855					860		
ATG Met	GAG Glu	ACC Thr	ATG Met	GGC Gly	ATC Ile	TAC Tyr	TTT Phe	GCA Ala	ACA Thr	CCA Pro	GAA Glu	TGG Trp	GTA Val	GCA Ala	CTC Leu	2761
			865					870					875			
AAT Asn	GGG Gly	CAC His	CGA Arg	GGG Gly	CCA Pro	AGC Ser	CCC Pro	GGC Gly	CAG Gln	CTA Leu	AAG Lys	TAC Tyr	TGG Trp	CAG Gln	AAC Asn	2809
		880					885						890			
ACA Thr	CGA Arg	GAA Glu	ATA Ile	CCG Pro	GAC Asp	CCA Pro	AAC Asn	GAG Glu	GAC Asp	TAT Tyr	CTA Leu	GAC Asp	TAC Tyr	GTG Val	CAT His	2857
	895					900					905					
GCA Ala	GAG Glu	AAG Lys	AGC Ser	CGG Arg	TTG Leu	GCA Ala	TCA Ser	GAA Glu	GAA Glu	CAA Gln	ATC Ile	CTA Leu	AGG Arg	GCA Ala	GCT Ala	2905
	910				915					920					925	
ACG Thr	TCG Ser	ATC Ile	TAC Tyr	GGG Gly	GCT Ala	CCA Pro	GGA Gly	CAG Gln	GCA Ala	GAG Glu	CCA Pro	CCC Pro	CAA Gln	GCT Ala	TTC Phe	2953
				930					935					940		

ATA GAC GAA GTT GCC AAA GTC TAT GAA ATC AAC CAT GGA CGT GGC CCA	3001
Ile Asp Glu Val Ala Lys Val Tyr Glu Ile Asn His Gly Arg Gly Pro	
945 950 955	
AAC CAA GAA CAG ATG AAA GAT CTG CTC TTG ACT GCG ATG GAG ATG AAG	3049
Asn Gln Glu Gln Met Lys Asp Leu Leu Leu Thr Ala Met Glu Met Lys	
960 965 970	
CAT CGC AAT CCC AGG CGG GCT CTA CCA AAG CCC AAG CCA AAA CCC AAT	3097
His Arg Asn Pro Arg Arg Ala Leu Pro Lys Pro Lys Pro Lys Pro Asn	
975 980 985	
GCT CCA ACA CAG AGA CCC CCT GGT CGG CTG GGC CGC TGG ATC AGG ACC	3145
Ala Pro Thr Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr	
990 995 1000 1005	
GTC TCT GAT GAG GAC CTT GAG TGAGGCTCCT GGGAGTCTCC CGACACCACC	3196
Val Ser Asp Glu Asp Leu Glu	
1010	
CGCGCAGGTG TGGACACCAA TTCGGCCTTA CAACATCCCA AATTGGATCC GTTCGCGGGT	3256
CCCCCT	3261

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1012 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met	Thr	Asn	Leu	Gln	Asp	Gln	Thr	Gln	Gln	Ile	Val	Pro	Phe	Ile	Arg
1				5				10						15	
Ser	Leu	Leu	Met	Pro	Thr	Thr	Gly	Pro	Ala	Ser	Ile	Pro	Asp	Asp	Thr
			20					25					30		
Leu	Glu	Lys	His	Thr	Leu	Arg	Ser	Glu	Thr	Ser	Thr	Tyr	Asn	Leu	Thr
		35					40					45			
Val	Gly	Asp	Thr	Gly	Ser	Gly	Leu	Ile	Val	Phe	Phe	Pro	Gly	Phe	Pro
	50					55					60				
Gly	Ser	Ile	Val	Gly	Ala	His	Tyr	Thr	Leu	Gln	Gly	Asn	Gly	Asn	Tyr
65					70					75					80

Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro Ala Ser Tyr
 85 90 95
 Asn Tyr Cys Arg Leu Val Ser Arg Ser Leu Thr Val Arg Ser Ser Thr
 100 105 110
 Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr Ile Asn Ala Val Thr
 115 120 125
 Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gly Leu
 130 135 140
 Met Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val
 145 150 155 160
 Gly Glu Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly
 165 170 175
 Tyr Val Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys
 180 185 190
 Met Val Ala Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile
 195 200 205
 Thr Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Pro Gly Gly
 210 215 220
 Val Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu
 225 230 235 240
 Ser Val Gly Gly Glu Leu Val Phe Gln Thr Ser Val His Gly Leu Val
 245 250 255
 Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Thr Val Ile
 260 265 270
 Thr Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn
 275 280 285
 Leu Met Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro
 290 295 300
 Ile Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln
 305 310 315 320
 Ala Gly Asp Gln Met Ser Trp Ser Ala Arg Gly Ser Leu Ala Val Thr
 325 330 335
 Ile His Gly Gly Asn Tyr Pro Gly Ala Leu Arg Pro Val Thr Leu Val
 340 345 350

Ala Tyr Glu Arg Val Ala Thr Gly Ser Val Val Thr Val Ala Gly Val
 355 360 365
 Ser Asn Phe Glu Leu Ile Pro Asn Pro Glu Leu Ala Lys Asn Leu Val
 370 375 380
 Thr Glu Tyr Gly Arg Phe Asp Pro Gly Ala Met Asn Tyr Thr Lys Leu
 385 390 395 400
 Ile Leu Ser Glu Arg Asp Arg Leu Gly Ile Lys Thr Val Trp Pro Thr
 405 410 415
 Arg Glu Tyr Thr Asp Phe Arg Glu Tyr Phe Met Glu Val Ala Asp Leu
 420 425 430
 Asn Ser Pro Leu Lys Ile Ala Gly Ala Phe Gly Phe Lys Asp Ile Ile
 435 440 445
 Arg Ala Ile Arg Arg Ile Ala Val Pro Val Val Ser Thr Leu Phe Pro
 450 455 460
 Pro Ala Ala Pro Leu Ala His Ala Ile Gly Glu Gly Val Asp Tyr Leu
 465 470 475 480
 Leu Gly Asp Glu Ala Gln Ala Ala Ser Gly Thr Ala Arg Ala Ala Ser
 485 490 495
 Gly Lys Ala Arg Ala Ala Ser Gly Arg Ile Arg Gln Leu Thr Leu Ala
 500 505 510
 Ala Asp Lys Gly Tyr Glu Val Val Ala Asn Leu Phe Gln Val Pro Gln
 515 520 525
 Asn Pro Val Val Asp Gly Ile Leu Ala Ser Pro Gly Val Leu Arg Gly
 530 535 540
 Ala His Asn Leu Asp Cys Val Leu Arg Glu Gly Ala Thr Leu Phe Pro
 545 550 555 560
 Val Val Ile Thr Thr Val Glu Asp Ala Met Thr Pro Lys Ala Leu Asn
 565 570 575
 Ser Lys Met Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln Pro
 580 585 590
 Pro Ser Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val
 595 600 605
 Tyr Gly Tyr Ala Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp
 610 615 620

Tyr Thr Val Val Pro Ile Asp Asp Val Trp Asp Asp Ser Ile Met Leu
 625 630 635 640
 Ser Lys Asp Pro Ile Pro Pro Ile Val Gly Asn Ser Gly Asn Leu Ala
 645 650 655
 Ile Ala Tyr Met Asp Val Phe Arg Pro Lys Val Pro Ile His Val Ala
 660 665 670
 Met Thr Gly Ala Leu Asn Ala Cys Gly Glu Ile Glu Lys Val Ser Phe
 675 680 685
 Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu Gly Leu Arg Leu Ala
 690 695 700
 Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp Ala Thr Phe
 705 710 715 720
 Ile Lys Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg Leu Pro Tyr
 725 730 735
 Leu Asn Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln Tyr His Leu
 740 745 750
 Ala Met Ala Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala
 755 760 765
 Val Arg Ala Met Glu Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser
 770 775 780
 Ala Leu Ser Val Phe Met Trp Leu Glu Glu Asn Gly Ile Val Thr Asp
 785 790 795 800
 Met Ala Asn Phe Ala Leu Ser Asp Pro Asn Ala His Arg Met Arg Asn
 805 810 815
 Phe Leu Ala Asn Ala Pro Gln Ala Gly Ser Lys Ser Gln Arg Ala Lys
 820 825 830
 Tyr Gly Thr Ala Gly Tyr Gly Val Glu Ala Arg Gly Pro Thr Pro Glu
 835 840 845
 Glu Ala Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys Met Glu Thr
 850 855 860
 Met Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His
 865 870 875 880
 Arg Gly Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu
 885 890 895

[illegible]

Age	Sex	Height (cm)	Weight (kg)	Body Fat (%)	Lean Body Mass (kg)	Basal Metabolic Rate (kcal/day)	Estimated Energy Requirement (kcal/day)
18	M	175	75	15	63.75	1750	2500
20	F	165	60	20	48.00	1350	1800
22	M	180	85	12	75.40	1950	2800
24	F	170	70	18	58.00	1550	2100
26	M	185	90	10	81.00	2100	3000
28	F	175	75	16	63.75	1750	2300
30	M	190	100	8	92.00	2300	3300
32	F	180	85	14	73.75	1900	2500
34	M	195	110	6	104.00	2500	3600
36	F	185	90	12	79.50	2050	2700
38	M	200	120	4	116.00	2700	3900
40	F	190	100	10	90.00	2150	2900
42	M	205	130	2	128.00	2900	4200
44	F	195	110	8	101.00	2300	3100
46	M	210	140	0	140.00	3100	4600
48	F	200	120	6	112.00	2500	3400
50	M	215	150	-2	152.00	3300	5000
52	F	205	130	4	126.00	2700	3700
54	M	220	160	-4	164.00	3500	5400
56	F	210	140	2	138.00	2900	4000
58	M	225	170	-6	176.00	3700	5800
60	F	215	150	0	150.00	3100	4300
62	M	230	180	-8	192.00	4000	6200
64	F	220	160	2	158.00	3300	4600
66	M	235	190	-10	200.00	4300	6600
68	F	225	170	4	163.00	3500	5000
70	M	240	200	-12	218.00	4700	7000
72	F	230	180	6	172.00	3700	5400
74	M	245	210	-14	226.00	5000	7400
76	F	235	190	8	171.00	3900	5800
78	M	250	220	-16	234.00	5300	7800
80	F	240	200	10	180.00	4100	6200

- | Age | Sex | Height (cm) | Weight (kg) | Body Mass Index (kg/m ²) | Waist Circumference (cm) | Waist-Hip Ratio | Trunk Fat (%) | Visceral Fat (cm) | Subcutaneous Fat (cm) | Visceral Fat Index (cm ²) | Subcutaneous Fat Index (cm ²) | Visceral Fat to Subcutaneous Fat Ratio |
|-------|-----|-------------|-------------|--------------------------------------|--------------------------|-----------------|---------------|-------------------|-----------------------|---------------------------------------|---|--|
| 20-29 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 30-39 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 40-49 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 50-59 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 60-69 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 70-79 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 80-89 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 90-99 | M | 175.0 | 75.0 | 24.5 | 95.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 20-29 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 30-39 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 40-49 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 50-59 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 60-69 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 70-79 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 80-89 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |
| 90-99 | F | 160.0 | 60.0 | 23.7 | 85.0 | 0.95 | 15.0 | 1.5 | 1.0 | 1.5 | 1.0 | 1.5 |

Age	Sex	Height (cm)	Weight (kg)	Body Mass Index (kg/m ²)	Waist Circumference (cm)	Waist-Hip Ratio	Trunk Fat (%)	Visceral Fat (cm)	Subcutaneous Fat (cm)	Visceral Fat Index (cm ²)	Subcutaneous Fat Index (cm ²)	Visceral Fat to Subcutaneous Fat Ratio
20-29	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
30-39	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
40-49	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
50-59	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
60-69	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
70-79	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
80-89	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
90-99	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
20-29	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
30-39	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
40-49	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
50-59	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
60-69	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
70-79	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
80-89	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
90-99	F	160.0	60.0	23.7	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5

- | Age | Sex | Height (cm) | Weight (kg) | Body Mass Index (kg/m ²) | Waist Circumference (cm) | Hip Circumference (cm) | Waist-Hip Ratio |
|---------|-----|-------------|-------------|--------------------------------------|--------------------------|------------------------|-----------------|
| 18-24 | M | 175.0 | 75.0 | 24.5 | 95.0 | 105.0 | 0.90 |
| 25-34 | F | 160.0 | 60.0 | 23.7 | 85.0 | 95.0 | 0.89 |
| 35-44 | M | 170.0 | 70.0 | 24.1 | 90.0 | 100.0 | 0.90 |
| 45-54 | F | 155.0 | 55.0 | 22.6 | 80.0 | 90.0 | 0.89 |
| 55-64 | M | 165.0 | 65.0 | 23.9 | 85.0 | 95.0 | 0.89 |
| 65-74 | F | 150.0 | 50.0 | 22.2 | 75.0 | 85.0 | 0.88 |
| 75-84 | M | 160.0 | 60.0 | 23.7 | 80.0 | 90.0 | 0.89 |
| 85-94 | F | 145.0 | 45.0 | 21.4 | 70.0 | 80.0 | 0.88 |
| 95-104 | M | 155.0 | 55.0 | 22.6 | 75.0 | 85.0 | 0.88 |
| 105-114 | F | 140.0 | 40.0 | 20.0 | 65.0 | 75.0 | 0.87 |
| 115-124 | M | 150.0 | 50.0 | 22.2 | 70.0 | 80.0 | 0.88 |
| 125-134 | F | 135.0 | 35.0 | 18.5 | 60.0 | 70.0 | 0.86 |
| 135-144 | M | 145.0 | 45.0 | 21.4 | 65.0 | 75.0 | 0.87 |
| 145-154 | F | 130.0 | 30.0 | 16.7 | 55.0 | 65.0 | 0.85 |
| 155-164 | M | 140.0 | 40.0 | 18.5 | 60.0 | 70.0 | 0.86 |
| 165-174 | F | 125.0 | 25.0 | 14.8 | 50.0 | 60.0 | 0.83 |
| 175-184 | M | 135.0 | 35.0 | 18.5 | 55.0 | 65.0 | 0.87 |
| 185-194 | F | 120.0 | 20.0 | 13.9 | 45.0 | 55.0 | 0.82 |
| 195-204 | M | 130.0 | 30.0 | 16.7 | 50.0 | 60.0 | 0.83 |
| 205-214 | F | 115.0 | 15.0 | 11.3 | 40.0 | 50.0 | 0.80 |
| 215-224 | M | 125.0 | 25.0 | 14.8 | 45.0 | 55.0 | 0.82 |
| 225-234 | F | 110.0 | 10.0 | 8.3 | 35.0 | 45.0 | 0.78 |
| 235-244 | M | 120.0 | 20.0 | 13.9 | 40.0 | 50.0 | 0.80 |
| 245-254 | F | 105.0 | 5.0 | 4.5 | 30.0 | 40.0 | 0.75 |
| 255-264 | M | 115.0 | 15.0 | 11.3 | 35.0 | 45.0 | 0.78 |
| 265-274 | F | 100.0 | 0.0 | 0.0 | 25.0 | 35.0 | 0.71 |
| 275-284 | M | 110.0 | 10.0 | 8.3 | 30.0 | 40.0 | 0.75 |
| 285-294 | F | 95.0 | -5.0 | -0.5 | 20.0 | 30.0 | 0.67 |
| 295-304 | M | 105.0 | 5.0 | 4.5 | 25.0 | 35.0 | 0.71 |
| 305-314 | F | 90.0 | -10.0 | -1.1 | 15.0 | 25.0 | 0.60 |
| 315-324 | M | 100.0 | 0.0 | 0.0 | 20.0 | 30.0 | 0.67 |
| 325-334 | F | 85.0 | -15.0 | -1.8 | 10.0 | 20.0 | 0.50 |
| 335-344 | M | 95.0 | -5.0 | -0.5 | 15.0 | 25.0 | 0.60 |
| 345-354 | F | 80.0 | -20.0 | -2.5 | 5.0 | 15.0 | 0.33 |
| 355-364 | M | 90.0 | -10.0 | -1.1 | 10.0 | 20.0 | 0.50 |
| 365-374 | F | 75.0 | -25.0 | -3.2 | 0.0 | 10.0 | 0.00 |
| 375-384 | M | 85.0 | -15.0 | -1.8 | 5.0 | 15.0 | 0.33 |
| 385-394 | F | 70.0 | -30.0 | -4.3 | -5.0 | 5.0 | -0.10 |
| 395-404 | M | 80.0 | -20.0 | -2.5 | 0.0 | 10.0 | 0.00 |
| 405-414 | F | 65.0 | -35.0 | -5.4 | -10.0 | 0.0 | -0.15 |
| 415-424 | M | 75.0 | -25.0 | -3.2 | -5.0 | 5.0 | -0.10 |
| 425-434 | F | 60.0 | -40.0 | -6.7 | -15.0 | -5.0 | -0.20 |
| 435-444 | M | 70.0 | -30.0 | -4.3 | -10.0 | 0.0 | -0.15 |
| 445-454 | F | 55.0 | -45.0 | -8.2 | -20.0 | -10.0 | -0.25 |
| 455-464 | M | 65.0 | -35.0 | -5.4 | -15.0 | -5.0 | -0.20 |
| 465-474 | F | 50.0 | -50.0 | -10.0 | -25.0 | -15.0 | -0.30 |
| 475-484 | M | 60.0 | -40.0 | -6.7 | -20. | | |

Age	Sex	Height (cm)	Weight (kg)	Body Mass Index (kg/m ²)	Waist Circumference (cm)	Hip Circumference (cm)	Waist-Hip Ratio
18-24	M	175.0	75.0	24.2	94.0	104.0	0.90
25-34	M	178.0	80.0	25.3	96.0	106.0	0.90
35-44	M	176.0	78.0	24.7	95.0	105.0	0.90
45-54	M	174.0	76.0	24.7	94.0	104.0	0.90
55-64	M	172.0	74.0	24.7	92.0	102.0	0.90
65-74	M	170.0	72.0	24.7	90.0	100.0	0.90
75-84	M	168.0	70.0	24.7	88.0	98.0	0.90
85-94	M	166.0	68.0	24.7	86.0	96.0	0.90
95-104	M	164.0	66.0	24.7	84.0	94.0	0.90
105-114	M	162.0	64.0	24.7	82.0	92.0	0.90
115-124	M	160.0	62.0	24.7	80.0	90.0	0.90
125-134	M	158.0	60.0	24.7	78.0	88.0	0.90
135-144	M	156.0	58.0	24.7	76.0	86.0	0.90
145-154	M	154.0	56.0	24.7	74.0	84.0	0.90
155-164	M	152.0	54.0	24.7	72.0	82.0	0.90
165-174	M	150.0	52.0	24.7	70.0	80.0	0.90
175-184	M	148.0	50.0	24.7	68.0	78.0	0.90
185-194	M	146.0	48.0	24.7	66.0	76.0	0.90
195-204	M	144.0	46.0	24.7	64.0	74.0	0.90
205-214	M	142.0	44.0	24.7	62.0	72.0	0.90
215-224	M	140.0	42.0	24.7	60.0	70.0	0.90
225-234	M	138.0	40.0	24.7	58.0	68.0	0.90
235-244	M	136.0	38.0	24.7	56.0	66.0	0.90
245-254	M	134.0	36.0	24.7	54.0	64.0	0.90
255-264	M	132.0	34.0	24.7	52.0	62.0	0.90
265-274	M	130.0	32.0	24.7	50.0	60.0	0.90
275-284	M	128.0	30.0	24.7	48.0	58.0	0.90
285-294	M	126.0	28.0	24.7	46.0	56.0	0.90
295-304	M	124.0	26.0	24.7	44.0	54.0	0.90
305-314	M	122.0	24.0	24.7	42.0	52.0	0.90
315-324	M	120.0	22.0	24.7	40.0	50.0	0.90
325-334	M	118.0	20.0	24.7	38.0	48.0	0.90
335-344	M	116.0	18.0	24.7	36.0	46.0	0.90
345-354	M	114.0	16.0	24.7	34.0	44.0	0.90
355-364	M	112.0	14.0	24.7	32.0	42.0	0.90
365-374	M	110.0	12.0	24.7	30.0	40.0	0.90
375-384	M	108.0	10.0	24.7	28.0	38.0	0.90
385-394	M	106.0	8.0	24.7	26.0	36.0	0.90
395-404	M	104.0	6.0	24.7	24.0	34.0	0.90
405-414	M	102.0	4.0	24.7	22.0	32.0	0.90
415-424	M	100.0	2.0	24.7	20.0	30.0	0.90
425-434	M	98.0	0.0	24.7	18.0	28.0	0.90
435-444	M	96.0	-2.0	24.7	16.0	26.0	0.90
445-454	M	94.0	-4.0	24.7	14.0	24.0	0.90
455-464	M	92.0	-6.0	24.7	12.0	22.0	0.90
465-474	M	90.0	-8.0	24.7	10.0	20.0	0.90
475-							

Age	Sex	Height (cm)	Weight (kg)	Body Fat (%)	Lean Body Mass (kg)	Basal Metabolic Rate (kcal/day)	Resting Metabolic Rate (kcal/day)	Physical Activity Level (kcal/day)	Total Daily Energy Expenditure (kcal/day)
20	M	175	75	15	63.75	1750	1925	1500	3375
25	F	160	55	20	44	1350	1450	1000	2400
30	M	180	85	18	69.75	1900	2050	1800	3850
35	F	165	60	22	46	1400	1500	1100	2500
40	M	170	70	20	56	1600	1750	1400	3050
45	F	155	50	25	37.5	1200	1300	900	2200
50	M	175	75	22	58.5	1650	1800	1500	3150
55	F	160	55	28	39.5	1150	1250	800	2000
60	M	170	70	25	52.5	1550	1650	1300	2850
65	F	150	45	30	31.5	1050	1150	700	1800
70	M	165	65	28	47	1450	1550	1200	2700
75	F	145	40	35	26	900	1000	600	1500
80	M	160	60	30	42	1350	1450	1100	2450
85	F	140	35	38	22.25	800	900	500	1300
90	M	155	55	35	35.75	1100	1200	800	1900
95	F	135	30	40	18	600	700	400	1100
100	M	150	50	38	32	1000	1100	700	1700

Age	Sex	Height (cm)	Weight (kg)	Body Mass Index (kg/m ²)	Waist Circumference (cm)	Waist-Hip Ratio	Trunk Fat (%)	Visceral Fat (cm)	Subcutaneous Fat (cm)	Visceral Fat Index (cm ²)	Subcutaneous Fat Index (cm ²)	Visceral Fat to Subcutaneous Fat Ratio
20-29	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
30-39	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
40-49	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
50-59	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
60-69	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
70-79	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
80-89	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
90-99	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
20-29	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
30-39	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
40-49	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
50-59	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
60-69	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
70-79	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
80-89	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
90-99	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5

Age	Sex	Height (cm)	Weight (kg)	Body Mass Index (kg/m ²)	Waist Circumference (cm)	Waist-Hip Ratio	Trunk Fat (%)	Visceral Fat (cm)	Subcutaneous Fat (cm)	Visceral Fat Index (cm ²)	Subcutaneous Fat Index (cm ²)	Visceral Fat to Subcutaneous Fat Ratio
20-29	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
30-39	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
40-49	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
50-59	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
60-69	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
70-79	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
80-89	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
90-99	M	175.0	75.0	24.5	95.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
20-29	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
30-39	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
40-49	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
50-59	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
60-69	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
70-79	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
80-89	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5
90-99	F	160.0	60.0	23.1	85.0	0.95	15.0	1.5	1.0	1.5	1.0	1.5

ACA	AAC	GAT	CGC	AGC	GAT	GAC	AAA	CCT	GCA	AGA	TCA	AAC	CCA	ACA	GAT	162
Thr	Asn	Asp	Arg	Ser	Asp	Asp	Lys	Pro	Ala	Arg	Ser	Asn	Pro	Thr	Asp	
			10					15					20			

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2827 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 112..2745

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGATACGATG	GGTCTGACCC	TCTGGGAGTC	ACGAATTAAC	GTGGCTACTA	GGGGCGATAC	60
CCGCCGCTGG	CTGCCACGTT	AGTGGCTCCT	CTTCTTGATG	ATTCTGCCAC	C ATG AGT	117
					Met Ser	1
GAC ATT TTC AAC AGT CCA CAG GCG CGA AGC ACG ATC TCA GCA GCG TTC	165					
Asp Ile Phe Asn Ser Pro Gln Ala Arg Ser Thr Ile Ser Ala Ala Phe						
	5 10 15					
GGC ATA AAG CCT ACT GCT GGA CAA GAC GTG GAA GAA CTC TTG ATC CCT	213					
Gly Ile Lys Pro Thr Ala Gly Gln Asp Val Glu Glu Leu Leu Ile Pro						
	20 25 30					
AAA GTT TGG GTG CCA CCT GAG GAT CCG CTT GCC AGC CCT AGT CGA CTG	261					
Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser Arg Leu						
	35 40 45 50					
GCA AAG TTC CTC AGA GAG AAC GGC TAC AAA GTT TTG CAG CCG CGG TCT	309					
Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Val Leu Gln Pro Arg Ser						
	55 60 65					
CTG CCC GAG AAT GAG GAG TAT GAG ACC GAC CAA ATA CTC CCA GAC TTA	357					
Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro Asp Leu						
	70 75 80					

GCA	TGG	ATG	CGA	CAG	ATA	GAA	GGG	GCT	GTT	TTA	AAA	CCC	ACT	CTA	TCT	405
Ala	Trp	Met	Arg	Gln	Ile	Glu	Gly	Ala	Val	Leu	Lys	Pro	Thr	Leu	Ser	
	85						90					95				
CTC	CCT	ATT	GGA	GAT	CAG	GAG	TAC	TTC	CCA	AAG	TAC	TAC	CCA	ACA	CAT	453
Leu	Pro	Ile	Gly	Asp	Gln	Glu	Tyr	Phe	Pro	Lys	Tyr	Tyr	Pro	Thr	His	
	100					105					110					
CGC	CCT	AGC	AAG	GAG	AAG	CCC	AAT	GCG	TAC	CCG	CCA	GAC	ATC	GCA	CTA	501
Arg	Pro	Ser	Lys	Glu	Lys	Pro	Asn	Ala	Tyr	Pro	Pro	Asp	Ile	Ala	Leu	
115					120					125					130	
CTC	AAG	CAG	ATG	ATT	TAC	CTG	TTT	CTC	CAG	GTT	CCA	GAG	GCC	AAC	GAG	549
Leu	Lys	Gln	Met	Ile	Tyr	Leu	Phe	Leu	Gln	Val	Pro	Glu	Ala	Asn	Glu	
				135					140					145		
GGC	CTA	AAG	GAT	GAA	GTA	ACC	CTC	TTG	ACC	CAA	AAC	ATA	AGG	GAC	AAG	597
Gly	Leu	Lys	Asp	Glu	Val	Thr	Leu	Leu	Thr	Gln	Asn	Ile	Arg	Asp	Lys	
			150					155					160			
CCC	TAT	GGA	AGT	GGG	ACC	TAC	ATG	GGA	CAA	GCA	ACT	CGA	CTT	GTG	GCC	645
Ala	Tyr	Gly	Ser	Gly	Thr	Tyr	Met	Gly	Gln	Ala	Thr	Arg	Leu	Val	Ala	
		165					170					175				
ATG	AAG	GAG	GTC	GCC	ACT	GGA	AGA	AAC	CCA	AAC	AAG	GAT	CCT	CTA	AAG	693
Met	Lys	Glu	Val	Ala	Thr	Gly	Arg	Asn	Pro	Asn	Lys	Asp	Pro	Leu	Lys	
	180					185					190					
CTT	GGG	TAC	ACT	TTT	GAG	AGC	ATC	GCG	CAG	CTA	CTT	GAC	ATC	ACA	CTA	741
Leu	Gly	Tyr	Thr	Phe	Glu	Ser	Ile	Ala	Gln	Leu	Leu	Asp	Ile	Thr	Leu	
195					200					205					210	
CCG	GTA	GGC	CCA	CCC	GGT	GAG	GAT	GAC	AAG	CCC	TGG	GTG	CCA	CTC	ACA	789
Pro	Val	Gly	Pro	Pro	Gly	Glu	Asp	Asp	Lys	Pro	Trp	Val	Pro	Leu	Thr	
				215					220					225		
AGA	GTG	CCG	TCA	CGG	ATG	TTG	GTG	CTG	ACG	GGA	GAC	GTA	GAT	GGC	GAC	837
Arg	Val	Pro	Ser	Arg	Met	Leu	Val	Leu	Thr	Gly	Asp	Val	Asp	Gly	Asp	
			230					235					240			
TTT	GAG	GTT	GAA	GAT	TAC	CTT	CCC	AAA	ATC	AAC	CTC	AAG	TCA	TCA	AGT	885
Phe	Glu	Val	Glu	Asp	Tyr	Leu	Pro	Lys	Ile	Asn	Leu	Lys	Ser	Ser	Ser	
	245						250					255				
GGA	CTA	CCA	TAT	GTA	GGT	CGC	ACC	AAA	GGA	GAG	ACA	ATT	GGC	GAG	ATG	933
Gly	Leu	Pro	Tyr	Val	Gly	Arg	Thr	Lys	Gly	Glu	Thr	Ile	Gly	Glu	Met	
	260					265					270					
ATA	GCT	ATA	TCA	AAC	CAG	TTT	CTC	AGA	GAG	CTA	TCA	ACA	CTG	TTG	AAG	981
Ile	Ala	Ile	Ser	Asn	Gln	Phe	Leu	Arg	Glu	Leu	Ser	Thr	Leu	Leu	Lys	
275					280					285					290	

CAA	GGT	GCA	GGG	ACA	AAG	GGG	TCA	AAC	AAG	AAG	AAG	CTA	CTC	AGC	ATG	1029
Gln	Gly	Ala	Gly	Thr	Lys	Gly	Ser	Asn	Lys	Lys	Lys	Leu	Leu	Ser	Met	
				295					300					305		
TTA	AGT	GAC	TAT	TGG	TAC	TTA	TCA	TGC	GGG	CTT	TTG	TTT	CCA	AAG	GCT	1077
Leu	Ser	Asp	Tyr	Trp	Tyr	Leu	Ser	Cys	Gly	Leu	Leu	Phe	Pro	Lys	Ala	
			310					315					320			
GAA	AGG	TAC	GAC	AAA	AGT	ACA	TGG	CTC	ACC	AAG	ACC	CGG	AAC	ATA	TGG	1125
Glu	Arg	Tyr	Asp	Lys	Ser	Thr	Trp	Leu	Thr	Lys	Thr	Arg	Asn	Ile	Trp	
		325					330					335				
TCA	GCT	CCA	TCC	CCA	ACA	CAC	CTC	ATG	ATC	TCC	ATG	ATC	ACC	TGG	CCC	1173
Ser	Ala	Pro	Ser	Pro	Thr	His	Leu	Met	Ile	Ser	Met	Ile	Thr	Trp	Pro	
	340					345					350					
GTG	ATG	TCC	AAC	AGC	CCA	AAT	AAC	GTG	TTG	AAC	ATT	GAA	GGG	TGT	CCA	1221
Val	Met	Ser	Asn	Ser	Pro	Asn	Asn	Val	Leu	Asn	Ile	Glu	Gly	Cys	Pro	
355					360					365					370	
TCA	CTC	TAC	AAA	TTC	AAC	CCG	TTC	AGA	GGA	GGG	TTG	AAC	AGG	ATC	GTC	1269
Ser	Leu	Tyr	Lys	Phe	Asn	Pro	Phe	Arg	Gly	Gly	Leu	Asn	Arg	Ile	Val	
				375					380					385		
GAG	TGG	ATA	TTG	GCC	CCG	GAA	GAA	CCC	AAG	GCT	CTT	GTA	TAT	GCG	GAC	1317
Glu	Trp	Ile	Leu	Ala	Pro	Glu	Glu	Pro	Lys	Ala	Leu	Val	Tyr	Ala	Asp	
			390					395					400			
AAC	ATA	TAC	ATT	GTC	CAC	TCA	AAC	ACG	TGG	TAC	TCA	ATT	GAC	CTA	GAG	1365
Asn	Ile	Tyr	Ile	Val	His	Ser	Asn	Thr	Trp	Tyr	Ser	Ile	Asp	Leu	Glu	
		405					410					415				
AAG	GGT	GAG	GCA	AAC	TGC	ACT	CGC	CAA	CAC	ATG	CAA	GCC	GCA	ATG	TAC	1413
Lys	Gly	Glu	Ala	Asn	Cys	Thr	Arg	Gln	His	Met	Gln	Ala	Ala	Met	Tyr	
	420					425					430					
TAC	ATA	CTC	ACC	AGA	GGG	TGG	TCA	GAC	AAC	GGC	GAC	CCA	ATG	TTC	AAT	1461
Tyr	Ile	Leu	Thr	Arg	Gly	Trp	Ser	Asp	Asn	Gly	Asp	Pro	Met	Phe	Asn	
435					440					445					450	
CAA	ACA	TGG	GCC	ACC	TTT	GCC	ATG	AAC	ATT	GCC	CCT	GCT	CTA	GTG	GTG	1509
Gln	Thr	Trp	Ala	Thr	Phe	Ala	Met	Asn	Ile	Ala	Pro	Ala	Leu	Val	Val	
				455					460					465		
GAC	TCA	TCG	TGC	CTG	ATA	ATG	AAC	CTG	CAA	ATT	AAG	ACC	TAT	GGT	CAA	1557
Asp	Ser	Ser	Cys	Leu	Ile	Met	Asn	Leu	Gln	Ile	Lys	Thr	Tyr	Gly	Gln	
			470					475					480			
GGC	AGC	GGG	AAT	GCA	GCC	ACG	TTC	ATC	AAC	AAC	CAC	CTC	TTG	AGC	ACG	1605
Gly	Ser	Gly	Asn	Ala	Ala	Thr	Phe	Ile	Asn	Asn	His	Leu	Leu	Ser	Thr	
		485					490					495				

CTA Leu 500	GTG Val 500	CTT Leu 500	GAC Asp 500	CAG Gln 500	TGG Trp 505	AAC Asn 505	TTG Leu 505	ATG Met 505	AGA Arg 510	CAG Gln 510	CCC Pro 510	AGA Arg 510	CCA Pro 510	GAC Asp 510	AGC Ser 510	1653
GAG Glu 515	GAG Glu 515	TTC Phe 515	AAA Lys 520	TCA Ser 520	ATT Ile 520	GAG Glu 520	GAC Asp 525	AAG Lys 525	CTA Leu 525	GGT Gly 525	ATC Ile 530	AAC Asn 530	TTT Phe 530	AAG Lys 530	ATT Ile 530	1701
GAG Glu 535	AGG Arg 535	TCC Ser 535	ATT Ile 535	GAT Asp 535	GAT Asp 540	ATC Ile 540	AGG Arg 540	GGC Gly 540	AAG Lys 540	CTG Leu 545	AGA Arg 545	CAG Gln 545	CTT Leu 545	GTC Val 545	CTC Leu 545	1749
CTT Leu 550	GCA Ala 550	CAA Gln 550	CCA Pro 550	GGG Gly 550	TAC Tyr 550	CTG Leu 555	AGT Ser 555	GGG Gly 555	GGG Gly 560	GTT Val 560	GAA Glu 560	CCA Pro 560	GAA Glu 560	CAA Gln 560	TCC Ser 560	1797
AGC Ser 565	CCA Pro 565	ACT Thr 565	GTT Val 570	GAG Glu 570	CTT Leu 570	GAC Asp 570	CTA Leu 570	CTA Leu 575	GGG Gly 575	TGG Trp 575	TCA Ser 575	GCT Ala 575	ACA Thr 575	TAC Tyr 575	AGC Ser 575	1845
AAA Lys 580	GAT Asp 580	CTC Leu 585	GGG Gly 585	ATC Ile 585	TAT Tyr 585	GTG Val 585	CCG Pro 590	GTG Val 590	CTT Leu 590	GAC Asp 590	AAG Lys 590	GAA Glu 590	CGC Arg 590	CTA Leu 590	TTT Phe 590	1893
TGT Cys 595	TCT Ser 595	GCT Ala 600	GCG Ala 600	TAT Tyr 600	CCC Pro 605	AAG Lys 605	GGA Gly 605	GTA Val 605	GAG Glu 605	AAC Asn 605	AAG Lys 610	AGT Ser 610	CTC Leu 610	AAG Lys 610	TCC Ser 610	1941
AAA Lys 615	GTC Val 615	GGG Gly 620	ATC Ile 620	GAG Glu 620	CAG Gln 625	GCA Ala 625	TAC Tyr 625	AAG Lys 625	GTA Val 625	GTC Val 625	AGG Arg 625	TAT Tyr 625	GAG Glu 625	GCG Ala 625	TTG Leu 625	1989
AGG Arg 630	TTG Leu 630	GTA Val 630	GGT Gly 630	GGT Gly 635	TGG Trp 635	AAC Asn 635	TAC Tyr 635	CCA Pro 635	CTC Leu 635	CTG Leu 640	AAC Asn 640	AAA Lys 640	GCC Ala 640	TGC Cys 640	AAG Lys 640	2037
AAT Asn 645	AAC Asn 645	GCA Ala 645	GGC Gly 645	GCC Ala 650	GCT Ala 650	CGG Arg 650	CGG Arg 650	CAT His 650	CTG Leu 650	GAG Glu 655	GCC Ala 655	AAG Lys 655	GGG Gly 655	TTC Phe 655	CCA Pro 655	2085
CTC Leu 660	GAC Asp 660	GAG Glu 660	TTC Phe 660	CTA Leu 665	GCC Ala 665	GAG Glu 665	TGG Trp 665	TCT Ser 665	GAG Glu 670	CTG Leu 670	TCA Ser 670	GAG Glu 670	TTC Phe 670	GGT Gly 670	GAG Glu 670	2133
GCC Ala 675	TTC Phe 675	GAA Glu 680	GGC Gly 680	TTC Phe 680	AAT Asn 680	ATC Ile 685	AAG Lys 685	CTG Leu 685	ACC Thr 685	GTA Val 685	ACA Thr 690	TCT Ser 690	GAG Glu 690	AGC Ser 690	CTA Leu 690	2181
GCC Ala 695	GAA Glu 695	CTG Leu 695	AAC Asn 695	AAG Lys 695	CCA Pro 695	GTA Val 700	CCC Pro 700	CCC Pro 700	AAG Lys 700	CCC Pro 705	CCA Pro 705	AAT Asn 705	GTC Val 705	AAC Asn 705	AGA Arg 705	2229

Recombinant birnavirus vaccine

The present invention is concerned with a birnavirus mutant, a vaccine comprising this mutant, a method for determining birnavirus infection in an animal, as well as with a test kit for carrying out this method.

Infectious bursal disease virus (IBDV) and Infectious pancreatic necrosis virus (IPNV) are members of the Birnaviridae family. Viruses in this family have a very similar genomic organisation and a similar replication cycle. The genomes of these viruses consist of 2 segments (A and B) of double-stranded (ds) RNA. The larger segment A encodes a polyprotein which is cleaved by autoproteolysis to form mature viral proteins VP2, VP3 and VP4 (Hudson, P.J. et al, Nucleic Acids Res., 14, 5001-50012, 1986; Dobos P., Annual review of fish diseases 5, 25-54, 1995). VP2 and VP3 are the major structural proteins of the virion. VP2 is the major host-protective immunogen of birnaviruses, and contains the antigenic regions responsible for the induction of neutralising antibodies. The VP4 protein appears to be a virus-coded protease that is involved in the processing of a precursor polyprotein of the VP2, VP3 and VP4 proteins. The larger segment A possesses also a second open reading frame (ORF), preceding and partially overlapping the polyprotein gene. This second open reading frame encodes a protein VP5 of unknown function that is present in IBDV infected cells (Mundt, E. et al., J. Gen. Virol., 76, 437-443, 1995).

The smaller segment B encodes VP1, a 90 kDa multifunctional protein with polymerase and capping enzyme activities (Spies, U. et al., Virus Res., 8, 127-140, 1987 and Spies, U. et al., J. Gen. Virol., 71, 977-981, 1990; Duncan R. et al., Virology 181, 541-552, 1991).

For IBDV, two serotypes exist, serotype 1 and 2. The 2 serotypes may be differentiated by virus neutralisation (VN) tests. Furthermore, subtypes of serotype 1 have been isolated. These so-called "variant" viruses of serotype 1 can be identified by cross-neutralisation tests (Diseases of Poultry, 9th edition, 1991, Wolfe Publishing Ltd, ISBN 0 7234 1706 7, Chapter 28, P.D. Lukert and Y.M. Saif, 648-663), a panel of monoclonal antibodies (Snyder, D.B. et al., Arch. Virol., 127, 89-101. 1992.) or RT-PCR (Jackwood, D.J., Proceedings of the International symposium on infectious bursal disease and chicken infectious anaemia, Rauischholzhausen, Germany, 155-161, 1994). Some of these subtypes of serotype 1 of IBDV have been described in literature for example: Classical, Variant-E, GLS, RS593 and DS326 strains (Van Loon, et

al. Proceedings of the International symposium on infectious bursal disease and chicken infectious anaemia, Rauschholzhausen, Germany, 179-187, 1994).

Infectious Bursal disease (IBD), also called Gumboro disease, is an acute, highly-contagious viral infection in chickens that has lymphoid tissue as its primary target with a selective tropism for cells of the bursa of Fabricius. The morbidity rate in susceptible flocks is high, with rapid weight loss and moderate mortality rates. Chicks that recover from the disease may have immune deficiencies because of the destruction of the bursa of Fabricius which is essential to the defence mechanism of the chicken. The IBD-virus causes severe immunosuppression in chickens younger than 3 weeks of age and induces bursal lesions in chicks up to 3 months old.

For many years the disease could be prevented by inducing high levels of antibodies in breeder flocks by the application of an inactivated vaccine, to chickens that had been primed with attenuated live IBDV vaccine. This has kept economic losses caused by IBD to a minimum. Maternal antibodies in chickens derived from vaccinated breeders prevents early infection with IBDV and diminishes problems associated with immunosuppression. In addition, attenuated live vaccines have also been used successfully in commercial chicken flocks after maternal antibodies had declined.

Recently, very virulent strains of IBDV have caused outbreaks of disease with high mortality in Europe. The current vaccination programs failed to protect chicks sufficiently. Vaccination failures were mainly due to the inability of live vaccines to infect the birds before challenge with virulent field virus.

Eradication of the disease by other preventative measures than vaccination has not been feasible, because the virus is widely spread and because with currently administered live attenuated or inactivated IBDV vaccines it is not possible to determine whether a specific animal is infected with an IBDV field virus or whether the animal was vaccinated with an IBDV vaccine. In order to be able to start an eradication control programme for IBDV it is highly desirable that the possibility exists to discriminate between animals vaccinated with an IBDV vaccine and those infected with a field virus so as to be able to take appropriate measures, i.e. remove infected flocks, to reduce spreading of the virulent field virus. The introduction of, for example, a serologically identifiable marker can be achieved by introducing

a mutation in genes encoding non-essential (glyco)proteins of the IBDV which still give rise to the production of antibodies in an infected host animal. A marker vaccine for Aujeszky's disease and companion diagnostic tests have proven their practical value in the control of this disease. Whereas such control programs for other viral infectious diseases in animals are under development, until the present invention a vaccine based on an IBDV vaccine strain which would fit in IBDV control programs has not been described yet. The main reason for this is that the prerequisites for the development for such an IBDV marker vaccine were not met. No permissive position or region in the genomic IBDV sequence, i.e. a position or region which can be used for the incorporation of the mutation without disrupting essential functions of IBDV, such as those necessary for infection and replication, have been identified yet. Moreover, such a non-essential region in the IBDV genome should encode a (glyco)protein which elicits a major serological response in an animal infected with wild-type IBDV, and such a region was not identified before.

The present inventors have unexpectedly found a non-essential gene within segment A of a birnavirus genome which can be mutated such that the resulting birnavirus mutant does not produce the native expression product of that gene. Moreover, it has been found that this birnavirus mutant can be used as a marker vaccine virus which allows to make a serological distinction between animals infected with wild-type birnavirus and animals immunised with a vaccine based on this birnavirus mutant.

The present invention provides a birnavirus mutant which is not able to produce a native VP5 protein as a result of a mutation in the VP5 gene of the birnavirus genome.

Preferably, the birnavirus mutant is an IBDV mutant or an IPNV mutant, the IBDV mutant being most preferred, in particular an IBDV mutant derived from a serotype 1 IBD virus is provided by the present invention.

The inventors have found that an IBDV mutant which is not able to produce the native VP5 protein is still able to infect cells and to replicate in these cells in vitro. It is demonstrated that the IBDV mutant according to the invention is replication competent in cell culture (Example 2). The VP5⁻ IBDV exhibits a delay in replication in chicken embryo cells as compared to the VP5⁺ parental virus, however, final yields of the virus are similar, i.e. about 10^{7.5} TCID₅₀/ml (Example 1). Moreover, it is demonstrated that the IBDV mutant is also able to

infect poultry and to replicate in the infected host animals in vivo, i.e. evidence is provided that the gene encoding the VP5 protein is a non-essential gene. Example 3 shows that the VP5⁻ IBDV can be re-isolated from organs of animals infected with the IBDV mutant and that the IBDV mutant induces a protective immune response in the infected animals.

Moreover, it has been established herein that part of the normal anti-IBDV immune response in poultry is directed to the VP5 region. This is rather surprising as the VP5 protein is considered to represent a non-structural viral protein (Mundt et al., J. Gen. Virol. 76, 437-443, 1995) and the immune response in an animal against a viral pathogen is usually elicited against the structural (glyco)proteins of the virus. These findings make the IBDV mutant and other birnavirus mutants according to the present invention a suitable vaccine candidate for a marker vaccine. Such a marker vaccine provides the possibility to determine whether animals are infected with a wild-type birnavirus, e.g. IBDV, or with a vaccine virus.

Additionally, it has been found that the VP5 protein is involved in the expression of virulence of the birnaviruses, in particular of IBDV, and that the inability of the virus mutants to produce the native VP5 protein leads to an attenuation of the virus.

With the term "which is not able to produce a native VP5 protein" is meant that the birnavirus mutant produces a polypeptide that can be distinguished by serological tests from the native VP5 protein, or does not produce a VP5 protein at all. For example, in the former case, the birnavirus mutant produces only a fragment of the native birnavirus VP5 protein which lacks one or more immunogenic epitopes.

Preferably, the birnavirus mutant according to the invention produces no VP5 protein upon infection of a host cell.

As described above, the genomic organisation of the birnaviruses is well established: the IBDV and IPNV genome comprises a large segment A and a smaller segment B. The segment A of IBDV comprises a large open reading frame (ORF) encoding a polyprotein of about 110 kDa (VP2-VP4-VP3). The gene encoding the VP5 protein is identified in the prior art, and defined herein, as the small ORF on segment A of the birnavirus genome which precedes and partially overlaps the polyprotein encoding ORF (Bayliss et al., J. Gen. Virol. 71, 1303-1312, 1990; Spies et al., J. Gen. Virol. 71, 977-981, 1990; Havarstein L.S. et al., J. Gen. Virology 71, 299-308; 1990; Dobos et al., 1995, supra; Figures 1-3 herein and SEQ ID No.'s 1-7). The mutation introduced in the VP5 gene is such that it does not prevent the expression of the polyprotein.

SEQ ID No. 1 comprises the full length cDNA nucleotide sequence of segment B of IBDV strain P2, as well as the amino acid sequence of the VP1 protein encoded by segment B (see also SEQ ID. No. 2). SEQ ID No. 3 and 5 depict the full length cDNA sequence of segment A of IBDV strain D78 and the coding region of the VP5 protein and the polyprotein, respectively. SEQ ID 3 and 4 also show the amino acid sequence of the D78 VP5 protein. SEQ ID No. 5 and 6 show the amino acid sequence of the polyprotein VP2-VP4-VP3 of D78. SEQ ID No. 7 shows the 5'-end of segment A of strain D78, including the mutations introduced in the VP5 coding region. SEQ ID No. 8 shows the nucleotide sequence of segment B of strain D78 and the amino acid sequence of the D78 VP1 protein. The genomic organisation of both segments is also shown in Figure 1.

The ORF coding for VP5 is conserved in all hitherto published segment A sequences. The IBDV ORF encodes 145 amino acids resulting in a calculated molecular mass of 16.5 kDa. The nucleotide sequence of the ORF encoding the VP5 protein of IBDV strain D78 used herein is shown in SEQ ID No. 3 and 4. Natural variations may exist between individual IBDV isolates. These natural variations result from small differences in the genomes of these viruses. The nucleotide sequence of the segment A, including the nucleotide sequence of the VP5 gene for many IBDV isolates have been described in the prior art (Vakharia et al., *Avian Diseases* 36, 736-742, 1992; Bayliss et al., *J. Gen. Virol.* 71, 1303-1314, 1990; Hudson et al., *Nuc. Acid Res.* 14, 5001-5012, 1986; Schnitzler et al., *J. Gen. Virol.* 47, 1563-1571, 1993; Kibenge et al., *J. Gen. Virol.* 71, 569-577, 1990 and *Virology* 184, 437-440, 1991; Mundt et al., *Virology* 209, 10-18, 1995; Lana et al., *Virus Genes* 6, 247-259, 1992; Vakharia et al., *Virus Res.* 31, 265-273, 1994; Brown et al., *Virus Res.* 40, 1-15, 1996). The amino acid sequence of the VP5 protein from serotype I IBDV strains display a homology of at least 95% with the VP5 amino acid sequence shown in SEQ ID No. 3 and 4, whereas the homology between serotype II VP5 sequence and the amino acid sequence shown in SEQ ID No. 3 and 4 is at least 75%. Therefore, a preferred IBDV mutant according to the present invention is an IBDV mutant wherein the mutation is introduced in the VP5 gene having a homology of at least 75%, in particular at least 95% on the amino acid sequence level with the VP5 amino acid sequence shown herein.

Preferably an IBDV mutant according to the present invention is derived from any of the classical or variant (e.g. variant E or GLS) IBDV vaccine strains, such as those currently used in the field. Such suitable IBDV strains include the IBDV vaccine strains present in the

commercially available vaccines: D78, PBG 98, LZ 228E, 89-03 (Intervet International B.V.), Bursine 2 (Fort Dodge Animal Health) and S 706 (Rhône Mérieux).

A particular preferred IBDV mutant according to the invention is derived from the D78 strain comprising a VP5 gene encoding a protein having the amino acid sequence shown in SEQ ID No. 3 and 4.

Alternatively, the parent birnavirus strain for the virus mutant according to the invention is a virulent birnavirus field strain. It is found herein that the VP5 protein is a factor associated with virulence, and that the absence of the native VP5 protein in a birnavirus results in an attenuated form of the virus.

Preferably the invention provides a birnavirus mutant which is not able to produce a native VP5 protein as a result of a mutation in the part of the VP5 gene which does not overlap with the large ORF encoding the polyprotein.

In particular, the birnavirus mutant according to the invention comprises a mutation in the 5'-end of the VP5 gene spanning nucleotides 1-30, preferably 1-20, more preferably 1-10. Most preferred is an birnavirus mutant having a mutation in nucleotides 1-3 of the VP5 gene.

A mutation is understood to be a change of the genetic information in the VP5 gene with respect to the genetic information present in this region of the genome of naturally occurring birnavirus producing native VP5 protein. The mutation is, for example, a nucleic acid substitution, deletion, insertion or inversion, or a combination thereof.

In a preferred embodiment of the present invention a birnavirus mutant is provided wherein the mutation is a substitution of one or more nucleotides. In particular, a nucleic acid substitution is introduced in the start codon, as a result of which the new codon encodes an amino acid different from methionine or represents a stop codon, preferably the nucleic acid substitution comprises at least two of the nucleotides of the start codon.

A further birnavirus mutant according to the invention comprises a substitution of one or more nucleotides in a codon(s) different from the start codon resulting in one or more stop codons, preferably in the 5'-end of the VP5 gene as defined above, if desired in addition to a substitution in the start codon as described above. Preferably, the birnavirus mutant comprises a stop codon in this region of the VP5 gene in each of the three reading frames.

Such a preferred birnavirus mutant may be an IBDV mutant having a mutation in the start codon, the fourth and the sixth codon of the VP5 gene, preferably resulting in the mutated codons shown in SEQ ID No. 7 and Figure 3.

Alternatively, a birnavirus mutant is provided wherein the mutation is a deletion. In particular, the deletion comprises less than 20, less than 10 or less than 5 nucleotides. Preferably, the deletion comprises a total number of nucleotides not dividable by three, resulting in a shift of the reading frame.

5 Preferably the deletion comprises one or more nucleotides of the start codon of the VP5 gene.

In an alternative embodiment of the present invention a birnavirus mutant is provided wherein the mutation comprises the insertion of a heterologous nucleic acid sequence in the birnavirus genome. A heterologous nucleic acid sequence is a nucleic acid sequence normally
10 not present at the specific insertion site of the particular virus species.

The heterologous nucleic sequence to be incorporated into the birnavirus genome is a nucleic acid fragment which either encodes a polypeptide or is a non-coding sequence. The nucleic acid fragment can be derived from any source, e.g. viral, eukaryotic, prokaryotic or synthetic, including oligonucleotides suitable for the interruption of the expression of the VP5
15 gene.

A suitable oligonucleotide for the interruption of the VP5 expression may comprise three translational stop codons in each of the possible reading frames in both directions, in addition to one or more appropriate restriction enzyme cleavage sites useful for the insertion of a second heterologous nucleic acid sequence. The length and nucleotide sequence of such a non-coding
20 heterologous nucleic acid sequence is not critical, but preferably varies between 8-50 nucleotides.

In a further embodiment of the present invention a birnavirus mutant is provided which can be used not only for the preparation of a vaccine against infection by a specific birnavirus, but also against other poultry or fish infectious diseases. For example, a vector vaccine based on
25 such an IBDV mutant offers the possibility to immunise against other avian pathogens by the expression of antigens of these avian pathogens within infected cells of the immunised host. Such an IBDV vector according to the present invention can be obtained by inserting a heterologous nucleic acid sequence encoding a polypeptide heterologous to the IBDV in the VP5 gene as defined herein.

30 The heterologous nucleic acid sequence may encode an antigen of an avian pathogen such as Newcastle disease virus, Infectious bronchitis virus, Marek's disease virus, avian

encephalomyelitis virus, avian reovirus, avian influenza virus, chicken anaemia virus, *Salmonella spp.*, *E.coli*, and *Eimeria spp.*

Furthermore, an IBDV mutant according to the invention comprises in addition to the mutation in the VP5 gene, a mutation in the VP2 gene, wherein this gene expresses a chimeric protein comprising neutralising epitopes of more than one antigenic type of IBDV (e.g. classic, Variant-E and/or GLS). Preferably, such a mutant comprises the relevant protective VP2 epitopes of a variant GLS strain and classic strain. In particular, the mutated VP2 gene is a GLS VP2 gene comprising a nucleic acid sequence fragment encoding the B69 epitope. The construction of such a mutated VP2 genes is described in Snyder et al., *Avian Diseases* 38, 701-707, 1994.

Furthermore, nucleic acid sequences encoding polypeptides for pharmaceutical or diagnostic applications, in particular immuno-modulators such as lymphokines, interferons or cytokines, may be incorporated into the VP5 gene. The heterologous nucleic acid sequence may also encode a screenable marker, such as *E. coli* β -galactosidase or *E. coli* β -glucuronidase.

The construction of birnavirus mutants, in particular of IBDV mutants according to the present invention can be achieved by means of the recently established infectious cRNA system for IBDV (Mundt and Vakharia, *Proc. Natl. Acad. Sci. USA* 93, 11131-11136, 1996). This reverse genetics system opens the possibility to introduce mutations in the RNA genome of the IBD virus, in particular in the VP5 gene. The most important step in this reverse genetics system is to provide full length cDNA clones of the segments A and B of IBD virus. cDNA constructs comprising the segment A or B, including the nucleotides of the 5'- and 3'- ends of both these segments can be generated according to the method described by Mundt and Vakharia (1996, supra). Additionally, these constructs comprise a RNA polymerase promoter operably linked to either of the segments. The promoter can be the promoter for the T7, SP6 or T3 polymerase, the T7 promoter being preferred. Mutations can be introduced into the VP5 gene by means of methods generally known in the art for this purpose. In particular, the mutation(s) are introduced by means of site directed mutagenesis.

For example, in a first step a cDNA fragment is provided comprising at least a substantial part of the VP5 gene. In the next step suitable primer pairs are designed and hybridised with the VP5 sequence containing fragment. The 5'-primer comprises in addition to sequences complementary to the VP5 sequence, nucleotides which harbour the desired mutation, e.g. a mutation which changes the ATG start codon to an AGG (arginine) codon. Moreover, the 5'-

primer is provided with an upstream nucleotide sequence representing a suitable restriction enzyme cleavage site which allows the restoring of the complete 5'-end non-coding sequence. Subsequently, the new mutated fragment is amplified using PCR and the new fragment is introduced in the starting sequence by replacing the native nucleic acid sequence using appropriate restriction enzymes. In the next step plus-sense transcripts of the segment A and B are generated in vitro with (T7) RNA polymerease, after which the synthetic transcripts are purified using conventional RNA purification techniques. The recombinant IBDV mutant according to the invention is obtained after transfection of suitable cells (e.g. VERO cells, QM-7 cells or CEC cells) with the synthetic RNA transcripts of both segments of the IBDV genome, if desired in the presence of transfection-enhancing compositions, such as Lipofectin. Finally the recombinant IBDV is harvested from the supernatant of the transformed cells.

Methods for introducing a mutation in the birnavirus genome are described herein, but are also generally used in the art (Mundt and Vakharia, 1996, *supra*; Current Protocols in Molecular Biology, eds.: F. M. Ausubel et al., Wiley N.Y., 1995 edition, pages 8.5.1.-8.5.9.)

Further to the unexpected finding by the present inventors that the VP5 ORF of IBDV is a non-essential region of the IBDV genome, it has also been found that an IBDV mutant according to the present invention is able to induce a protective immune response, i.e. animals immunised with a vaccine comprising the IBDV mutant are protected against virulent challenge. Moreover, it has been found that anti-sera of animals infected with naturally occurring IBDV comprise antibodies directed to the non-structural VP5 protein and that these antisera can be distinguished from anti-sera derived from animals infected with an IBDV mutant according to the present invention. In addition, it has been found that the IBDV mutant as described above is attenuated if compared with the parent IBD virus which is able to produce the native VP5 protein.

Therefore, another aspect of this invention is a vaccine for use in the protection of animals against birnavirus infection comprising the birnavirus mutant as characterised above, together with a pharmaceutical acceptable carrier or diluent. In particular, the vaccine according to the invention is a vaccine for use in the protection of poultry against infectious bursal disease comprising the IBDV mutant described above.

The birnavirus mutant according to the present invention can be incorporated into the vaccine as live or inactivated virus.

A vaccine according to the invention can be prepared by conventional methods such as for example commonly used for the commercially available live- and inactivated IBDV vaccines. Briefly, a susceptible substrate is inoculated with an IBDV mutant according to the invention and propagated until the virus replicated to a desired infectious titre after which IBDV containing material is harvested.

Every substrate which is able to support the replication of IBD viruses can be used in the present invention, including primary (avian) cell cultures, such as chicken embryo fibroblast cells (CEF) or chicken kidney cells (CK), mammalian cell lines such as the VERO cell line or the BGM-70 cell line, or avian cell lines such as QT-35, QM-7 or LMH. Usually, after inoculation of the cells, the virus is propagated for 3-10 days, after which the cell culture supernatant is harvested, and if desired filtered or centrifuged in order to remove cell debris.

Alternatively, the IBDV mutant is propagated in embryonated chicken eggs. In particular, the substrate on which these IBD viruses are propagated are SPF embryonated eggs. Embryonated eggs can be inoculated with, for example 0.2 ml IBDV mutant containing suspension or homogenate comprising at least 10^2 TCID₅₀ per egg, and subsequently incubated at 37 °C. After about 2-5 days the IBD virus product can be harvested by collecting the embryo's and/or the membranes and/or the allantoic fluid followed by appropriate homogenising of this material. The homogenate can be centrifuged thereafter for 10 min at 2500 x g followed by filtering the supernatant through a filter (100 µm).

The vaccine according to the invention containing the live virus can be prepared and marketed in the form of a suspension or in a lyophilised form and additionally contains a pharmaceutically acceptable carrier or diluent customary used for such compositions. Carriers include stabilisers, preservatives and buffers. Suitable stabilisers are, for example SPGA, carbohydrates (such as sorbitol, mannitol, starch, sucrose, dextran, glutamate or glucose), proteins (such as dried milk serum, albumin or casein) or degradation products thereof. Suitable buffers are for example alkali metal phosphates. Suitable preservatives are thimerosal, merthiolate and gentamicin. Diluents include water, aqueous buffer (such as buffered saline), alcohols and polyols (such as glycerol).

If desired, the live vaccines according to the invention may contain an adjuvant. Examples of suitable compounds and compositions with adjuvant activity are the same as mentioned below.

Although administration by injection, e.g. intramuscular, subcutaneous of the live vaccine according to the present invention is possible, the vaccine is preferably administered by the inexpensive mass application techniques commonly used for IBDV vaccination. For IBDV vaccination these techniques include drinking water and spray vaccination.

Alternative methods for the administration of the live vaccine include in ovo, eye drop and beak dipping administration.

In another aspect of the present invention a vaccine is provided comprising the birnavirus mutant in an inactivated form. The major advantage of an inactivated vaccine is the extremely high levels of protective antibodies of long duration that can be achieved.

The aim of inactivation of the viruses harvested after the propagation step is to eliminate reproduction of the viruses. In general, this can be achieved by chemical or physical means. Chemical inactivation can be effected by treating the viruses with, for example, enzymes, formaldehyde, β -propiolactone, ethylene-imine or a derivative thereof. If necessary, the inactivating compound is neutralised afterwards. Material inactivated with formaldehyde can, for example, be neutralised with thiosulphate. Physical inactivation can preferably be carried out by subjecting the viruses to energy-rich radiation, such as UV light or γ -rays. If desired, after treatment the pH can be adjusted to a value of about 7.

A vaccine containing the inactivated birnavirus mutant can, for example comprise one or more of the above-mentioned pharmaceutically acceptable carriers or diluents suited for this purpose.

Preferably, an inactivated vaccine according to the invention comprises one or more compounds with adjuvant activity. Suitable compounds or compositions for this purpose include aluminium hydroxide, -phosphate or -oxide, oil-in-water or water-in-oil emulsion based on, for example a mineral oil, such as Bayol F® or Marcol 52® or a vegetable oil such as vitamin E acetate, and saponins.

The vaccine according to the invention comprises an effective dosage of the birnavirus mutant as the active component, i.e. an amount of immunising birnavirus material that will induce immunity in the vaccinated birds against challenge by a virulent virus. Immunity is defined herein as the induction of a significant higher level of protection in a population of birds after vaccination compared to an unvaccinated group.

Typically, the live vaccine according to the invention can be administered in a dose of 10^2 - 10^9 TCID₅₀ infectious dose₅₀ (TCID₅₀) per animal, preferably in a dose ranging from 10^5 -

10^{70} TCID₅₀, and an inactivated vaccines may contain the antigenic equivalent of 10^5 - 10^9 TCID₅₀ per animal.

Inactivated vaccines are usually administered parenterally, e.g. intramuscularly or subcutaneously.

Although, the IBDV vaccine according to the present invention may be used effectively in chickens, also other poultry such as turkeys, guinea fowl and partridges may be successfully vaccinated with the vaccine. Chickens include broilers, reproduction stock and laying stock.

The age of the animals receiving a live or inactivated vaccine according to the invention is the same as that of the animals receiving the conventional live- or inactivated IBDV vaccines. For example, broilers (free of maternally derived antibodies-MDA) may be vaccinated at one-day-old, whereas broilers with high levels of MDA are preferably vaccinated at 2-3 weeks of age. Laying stock or reproduction stock with low levels of MDA may be vaccinated at 1-10 days of age followed by booster vaccinations with inactivated vaccine on 6-8 and 16-20 weeks of age.

The invention also includes combination vaccines comprising, in addition to the IBDV or IPNV mutant according to the invention, one or more immunogens derived from other pathogens infectious to poultry or fish, respectively.

Preferably, the combination vaccine additionally comprises one or more vaccine strains of infectious bronchitis virus (IBV), Newcastle disease virus (NDV), egg drop syndrome (EDS) virus, turkey rhinotracheitis virus (TRTV) or reovirus.

In addition to a marker vaccine for birnaviruses, the availability of an appropriate diagnostic test is an essential requirement for the application of a birnavirus eradication control programme. Such a diagnostic test is provided herein and comprises a method for determining IBDV infection in poultry and IPNV infection in fish, i.e. it provides a method for distinguishing an animal in the field vaccinated with a vaccine as described above, from an animal infected with a naturally-occurring IBDV or IPNV.

Therefore, the present invention provides a method for the detection of birnavirus infection, in particular for the detection of IBDV infection in an animal comprising the step of examining a sample of the animal for the presence of VP5 antibodies or antigens. The animal is an animal from the field and is in particular an avian species, preferably a chicken. The sample

coming from the animal may be any sample in which IBDV antibodies or antigens are present, e.g. a blood, serum or tissue sample, the serum sample being preferred.

A preferred method for determining birnavirus infection in an animal is a method for the detection of antibodies against the VP5 protein, comprising the steps of:

- (i) incubating a sample suspected of containing anti-birnavirus antibodies, with VP5 antigen,
- (ii) allowing the formation of antibody-antigen complex, and
- (ii) detecting the presence of the antibody-antigen complex.

The design of this immunoassay may vary. For example, the immunoassay may be based upon competition or direct reaction. Furthermore, protocols may use solid supports or may use cellular material. The detection of the antibody-antigen complex may involve the use of labelled antibodies; the labels may be, for example, enzymes, fluorescent-, chemiluminescent-, radio-active- or dye molecules.

Suitable methods for the detection of the VP5 antibodies in the sample include the enzyme-linked immunosorbent assay (ELISA), immunofluorescent test (IFT) and Western blot analysis.

In an exemplifying ELISA, the wells of a polystyrene micro-titration plate are coated with VP5 antigen. Next, the wells of the coated plates are filled with chicken serum and serial dilutions are made. After incubation, chicken anti-VP5 protein serum antibodies are determined by detecting antibody (monoclonal or polyclonal) with the same specificity as the coated one, but which is labelled (e.g. with biotin). The labelled antibody will occupy the free antigens that have not been occupied by anti-VP5 antibodies in the chicken serum. For example, horse radish peroxidase coupled to avidin may be added and the amount of peroxidase is measured by an enzymatic reaction. If no antibodies against VP5 are present in the chicken serum sample then a maximum absorption is obtained. If the serum contains many antibodies against VP5 then a low absorption is expected. Alternatively, after the incubation with chicken serum, the amount of antibodies present in the serum that bound to the VP5 antigen may be determined directly by using an anti-chicken conjugate followed by the enzymatic reaction.

In a sandwich ELISA the wells of a polystyrene micro-titration plate can be coated with a monoclonal antibody directed against the VP5 protein. Next, the wells of these coated plates are incubated with VP5 antigen. After the antigen is captured, the wells are filled with the chicken serum and serial dilutions are made. Subsequently, the protocol as described above may be

followed. This test can also be carried out by using polyclonal serum against VP5 instead of the coated monoclonal antibodies.

In another diagnostic test (Western blot analysis), the VP5 antigen (containing) material is subjected to SDS-PAGE. Next, the separated proteins are electroblotted onto nitro-cellulose membrane. Thereafter, the membranes can be cut into lanes and the lanes are incubated with the chicken serum. The presence of VP5 antibodies in the sample can be determined by examination whether antibodies bound to the VP5 antigen, for example by using an anti-chicken conjugate followed by an enzymatic reaction. If antibodies against VP5 are present then a band at about 17 kDa is identifiable.

The VP5 antigen may be any VP5 protein (fragment) comprising material which allows the formation of the VP5 antigen-VP5 antibody complex. Preferably, the VP5 antigen comprises the expression product of a conventional recombinant host cell or virus, e.g. such as E.coli expressed VP5 (Mundt et al., J. Gen. Virol. 76, 437-443, 1995) or baculovirus expressed protein (Vakharia et al., Vaccine 12, 452-456, 1994; Vakharia et al., J. Gen Virol. 74, 1201-1206, 1993). In a further embodiment of the present invention a diagnostic test kit is provided which is suitable for performing the diagnostic test according to the invention as described above.

In particular, a diagnostic test kit is provided which comprises in addition to the components usually present, the VP5 antigen (if desired coated onto a solid phase) as the immunological reagent. Other components usually present in such a test kit include, biotin or horseradish peroxidase conjugated antibodies, enzyme substrate, washing buffer etc.

To determine birnavirus VP5 antigen in a test sample from an animal in the field, VP5-specific antibodies are used as the immunological reagent, preferably fixed to a solid phase. The test sample is added, and after an incubation time allowing formation of the antibody-antigen complex, a second labelled antibody may be added to detect the complex.

EXAMPLES

Example 1.

Construction and analysis of recombinant VP5⁻ IBD virus

Construction of full length VP5⁻ clone of IBDV segment A.

To construct a VP5-negative IBDV, the *EcoRI* site immediately following the 3'-end of the full length cDNA of strain D78 segment A (pUC19FLAD78; Mundt and Vakharia, Proc. Natl. Acad. Sci. USA 93, 11131-11136, 1996) was deleted. An *EcoRI* - *KpnI* fragment containing the T7 polymerase binding site followed by the complete segment A sequence was excised and inserted into *EcoRI* - *KpnI* cleaved vector pUC18 after inactivation of the unique *NdeI* within the vector sequence resulting in plasmid pAD78/EK. Thereafter, the genomic region encompassing the initiation codon for VP5 was amplified in two pieces using primers A1F5' and VP5MutR, and VP5MutF and A2R, respectively (see Table 1 for sequence and location of primers). PCR fragments were cloned separately and were subsequently fused via a unique *AflIII* site which had been created by mutations within respective primers (see Fig. 2). An *EcoRI* - *NdeI* fragment containing the T7 polymerase binding site, and the 5'-part of segment A including the introduced mutations was excised and used to substitute the wild-type *EcoRI* - *NdeI* fragment in pAD78/EK to yield plasmid pAD78/VP5⁻. Of the three mutations introduced one altered the initiation methionine codon for VP5 into an arginine codon (Fig. 2).

Table 1: Sequence of oligonucleotide primers used for generating mutant constructs.

^a Nucleotide sequence	Orientation	Designation	Nucleotide no.
<u>AGAGAATTCTAATACGACTCACTATAGGA</u> <u>TACGATCGGTCTGAC</u>	+	A1F5'	1-18
<u>TGGGCCTGTCACGTGCTGTCACATGT</u>	-	A2R	716 - 740
<u>CATTGCTCTGCAGTGTGTAGTGAGC</u>	-	A3R	338 - 362
<u>CTACAACGCTATCCTTAAGGGTTAGTA</u> <u>GAG</u>	+	VP5MutF	80 - 109
<u>CTCTACTAACCCTTAAGGATAGCGTTGT</u> <u>AG</u>	-	VP5MutR	80 - 109

- a) Underlined nucleotides denote virus specific nucleotides. T7 promotor sequences are marked in italics. Mutated nucleotides are bold and orientation of the primer is shown for sense (+) and antisense (-). Primer positions are given according to the published sequence of serotype I strain P2 (Mundt et al., Virology 209, 209-218, 1995).

Virus recovery from cRNA. For *in vitro* transcription of RNA plasmids pAD78/EK, pAD78/VP5⁻ and pBP2 (Fig. 2) were linearized by cleavage with *Bsr*GI and *Pst*I, respectively. Treatment of linearized DNA, transcription and purification of RNA, and transfection were carried out as described by Mundt and Vakharia (1996, supra) with the exception that secondary CEC were used for the transfection experiments. Three days after transfection a CPE was visible in CEC. Cells were freeze/thawed, centrifuged at 700 x g to eliminate cellular debris, and the resulting supernatants were filtrated through 0.45 µm filters and stored at -20°C. For the transfection experiments full length cDNA clones of segment A of strain D78 capable of expressing (pAD78/EK) or unable to express VP5 (pAD78/VP5⁻) were transcribed into synthetic RNA and cotransfected with segment B full length cRNA into CEC. Resulting virus progeny IBDV/EK and IBDV/VP5⁻ was further characterised.

Analysis of transfection progeny by immunofluorescence and Radioimmunoprecipitation assay (RIPA). VP5 was expressed in E.coli as described in Mundt et al. (J. Gen. Virol. 76, 437-443, 1995). Rabbit monospecific polyclonal anti serum and mouse monoclonal antibodies against VP5 were prepared according to standard protocols. Vero cells infected with IBDV/VP5⁻, IBDV/EK, and non-infected cells, respectively, were incubated with rabbit anti-IBDV serum, rabbit anti-VP5 serum and with anti-VP5 mAb DIE 7, and stained with fluoresceine-conjugated secondary antibodies. Both antisera and the monoclonal antibody recognised IBDV antigens in the cytoplasm of IBDV/EK infected cells. In contrast, whereas the anti-IBDV serum readily detected viral antigens in IBDV/VP5⁻ infected cells, neither the monospecific anti VP5-serum nor the monoclonal anti-VP5 antibody exhibited specific reactivity. None of these immunological reagents reacted with non-infected controls.

To analyse viral proteins expressed during replication lysates of radioactively labelled CEC infected with IBDV/VP5⁻ (Fig 4, lanes 1-3) and IBDV/EK (Fig. 4, lanes 4-6) were immunoprecipitated with rabbit anti-IBDV serum, rabbit anti-VP5 serum and mAb DIE 7. Non-infected CEC were used as control (Fig. 4, lanes 7-9). IBDV/EK (lane 4) as well as IBDV/VP5⁻ (lane 1) infected CEC showed viral proteins VP2, VP3, and VP4 after precipitation with rabbit anti-IBDV serum. The rabbit anti-VP5 serum (lane 5) and mAb DIE 7 (lane 6) precipitated VP5 with a molecular mass of 21 kDa only from IBDV/EK infected cells. No specific reactivity was detectable in IBDV/VP5⁻ infected CEC after precipitation with rabbit-anti VP5 (lane 2) as well as the VP5 specific mAb DIE 7 (lane 3). Non-infected CEC showed no specific reactivity (lanes 7-9).

Replication of IBDV/VP5⁻ in CEC. To assay replication of IBDV/VP5⁻ in more detail one step growth was analysed (Fig. 5). Confluent secondary CEC were infected with IBDV/EK and IBDV/VP5⁻ with $10^{7.2}$ TCID₅₀, respectively. Immediately after overlaying the infected cells with 5 ml growth medium, supernatant from one infected CEC tissue plate of each virus was removed and stored at -20°C (0 h p.i.). Remaining tissue culture plates were further incubated and 4h, 8h, 16h, 24h, and 48h p.i. supernatants were removed and stored at -20°C. Supernatants were centrifuged and titrated according to standard methods. The TCID₅₀ at the different time points after infection showed that the VP5 expressing virus (IBDV/EK) replicated faster than the virus mutant lacking VP5 (IBDV/VP5⁻). 16 h after infection IBDV/EK showed a 100-fold higher than IBDV/VP5⁻ (Fig. 5). However, at 48 h p.i. IBDV/VP5⁻ reached a titre of $10^{7.2}$ TCID₅₀/ml which was similar to IBDV/EK ($10^{7.45}$ /ml)

Preparation of recombinant IBDV VP5⁻-2. Plasmid pAD78/VP5⁻-2 was prepared by techniques similar to those described above. The nucleotide sequence of part of the mutated VP5 gene is shown in SEQ ID No. 7 and Figure 3. A restriction enzyme fragment harbouring the mutations was used to substitute the wild-type *EcoRI* - *NdeI* fragment in pAD78/EK. An outline of the protocol for the preparation of the recombinant plasmid is shown in Figure 3. The organisation of pBD78 is also depicted in Figure 3. The recombinant virus was prepared as described above, except for the fact that segment B of strain D78 (SEQ ID No. 8) was used and QM-7 cells were used for the transfection experiment.

Example 2

Identification of VP5 protein in different IBDV strains

5 Different strains of IBDV were investigated for the expression of the VP5-gene. This was done by making use of the immuno-fluorescence technique (IFT). Chicken embryo fibroblasts grown in microtiterplates were infected with different IBDV strains. Three to 5 days after incubation at 37°C cells were fixed with 70% ethanol, then treated with polyclonal rabbit anti IBDV serum (R1928), polyclonal rabbit anti VP5 serum (R α VP5) or monoclonal antibody directed against VP5 (DIE7), respectively. Binding of the poly- or monoclonal antibodies to the
10 different IBDV strains was visualised by making use of a fluorescence labelled conjugate (goat-anti-rabbit or goat-anti-mouse). The results are shown in Table 2:

15 **Table 2:** Identification of different sero- and subtypes of IBDV strains. Determination of the presence of VP5 proteins.

IBDV-serotype	IBDV-subtype	IBDV-strain	R1928	R α VP5	DIE7
I	Classical	D78	+	+	+
I	Classical	228TC	+	+	+
I	Classical	PBG98	+	+	+
I	Classical	Ram0404	+	+	+
I	Classical	IBDV/EK	+	+	+
I	Classical	IBDV/VP5 ⁻	+	-	-
I	GLS	GLS	+	+	+
I	Variant-E	8903	+	+	+
II	TY89	TY89	+	+	+

From these data it can be concluded that the different strains of IBDV belonging to different sero- and subtypes do express the VP5-gene. Furthermore, the recombinant VP5⁻

IBDV vaccine strain can be differentiated from field and vaccine viruses, thereby enabling the recombinant VP5⁻ virus to be used as a marker vaccine.

Example 3

In vivo testing of the recombinant VP5⁺ and VP5⁻ IBDV vaccines in comparison with a commercial available live IBDV vaccine.

Preparation of IBDV vaccine. Primary chicken embryo fibroblast (CEF) cells were prepared at a final concentration of 2×10^6 /ml. The cells were cultured in Eagles minimum essential medium containing 5% fetal calf serum. To 25 ml of this cell suspension 0.1 ml IBDV/EK or IBDV/VP5⁻ virus (having an infectious titre of about $3.0 \log_{10}$ TCID₅₀/ml) was added. After incubation for 5 days in a high-humidity incubator at 37°C, the total suspension was used in the animal experiment without further purification. The infectious titre of the supernatant was $10^{7.1}$ TCID₅₀/ml.

Animal experiment. In this study the potency of different vaccines (VP5 positive strain IBDV/EK and a VP5 negative strain IBDV/VP5⁻, and the commercial available IBDV vaccine Nobilis strain D78, Intervet International B.V., NL) was investigated. SPF chicks of 3 weeks old were treated as indicated in the treatment schedule.

Treatment Schedule:

Days after vaccination	Groups			
	1	2	3	4
00	IBDV/EK	IBDV/VP5 ⁻	D78	-
03	x	x1	x	x
07	x,bl	x1,bl	x,b	x,bl
14	x,bl	x,bl	x,bl	x,bl
20	x,bl	x,bl	x,bl	x,bl
21	ch	ch	ch	ch
24	x	x	x	x
31	+	+	+	+

VP5⁺ Bursal disease vaccination with VP5 positive vaccine clone, eye-drop route, dose $10^{4.6}$ TCID₅₀/animal, 0.1 ml/animal.

VP5⁻ Bursal disease vaccination with VP5 negative vaccine clone, eye-drop route, dose $10^{5.9}$ TCID₅₀/animal, 0.1 ml/animal.

D78 Bursal disease vaccination with IBDV VACCINE NOBILIS STRAIN D78, eye-drop route, one field dose.

ch Challenge with Bursal disease virus, Farragher strain F52/70, eye-drop route, dose $10^{2.0}$ CID₅₀/animal, 0.1 ml/animal.

bl Serological examination; VN-test and/or Western blotting.

x Histological examination (H.E. staining) and MCA-8 ELISA on bursae.

x1 Histological examination (H.E. staining) and MCA-8 ELISA on bursae and reisolation of virus from bursa of Fabricius.

+ Clinical examination and after 10 days histological examination of the bursa.

Detection of virus in the bursa of Fabricius.

Three, 7, 14 and 20 days after eye-drop vaccination, animals were sacrificed and blood and bursae obtained. The presence of virus in the bursa was determined with an enzyme-linked immunosorbent assay (ELISA) making use of the monoclonal antibody 8 (MAB-8). MAB-8 is directed specifically against IBDV. Data are depicted in Table 3.

Furthermore, 3 and 7 days after vaccination, bursae from animals of group 2 were investigated for the presence of the recombinant VP5⁻ virus. For that purpose bursae were homogenised and cultured on chicken embryo fibroblasts. The presence of the VP5⁻ virus was determined by IFT using polyclonal rabbit sera against IBDV or VP5 or monoclonal antibodies against VP5. From 13 out of 15 bursae (87%) investigated, VP5⁻ virus could be reisolated and identified (positive for R1928 and negative for RαVP5 and DIE7). This indicates that the virus upon animal passage is still VP5⁻, indicating that the virus is stable and does not revert to VP5⁺. Furthermore, by using the different poly- and monoclonal antibodies VP5⁻ vaccine virus can be discriminated from all other vaccine and/or field IBDV viruses. Therefore, the VP5⁻ vaccine may be used as a marker vaccine.

Three days after challenge no virus could be detected in groups 1, 2 and 3 with the MCA-8 ELISA. In contrast, all animals of group 4 (non-vaccinated control group) contained challenge

virus in the bursa of Fabricius, 3 days after challenge. The results show that animals vaccinated with recombinant VP5⁺ (group 1), recombinant VP5⁻ (group 2) and IBDV vaccine Nobilis D78 (group 3) were protected against severe challenge:

5 **Table 3:** Individual data for detection of virus in the bursa of Fabricius with the MCA-8 ELISA at different days after vaccination or challenge.

	Days after vaccination→				Days after challenge	
	3	7	14	20	3	
Group↓	Virus detection by ELISA↓					Protection↓
1 VP5 ⁺	2/8*	1/7	0/2	0/3	0/5	100%
2 VP5 ⁻	0/8	0/7	0/2	0/3	0/5	100%
3 D78	1/8	6/7	0/2	0/3	0/5	100%
4 -	0/8	0/7	0/2	0/3	5/5	0%

*Number of positive bursae per total number tested.

Detection of lesions in the bursa of Fabricius.

The microscopic average lesion score induced by the different IBDV (recombinant) vaccines or the challenge virus are depicted in Table 4.

15 Before challenge, animals vaccinated with the recombinant VP5⁺ IBDV vaccine (group 1) or vaccinated with IBDV vaccine Nobilis D78 (group 3) showed mild to moderate lesions in the bursa. Three days after challenge only chronic lesions were observed in the bursa of Fabricius, indicating that the animals of groups 1 and 3 were protected against challenge. Furthermore, 10 days after challenge only very mild lesions (0-20% lymphocytic depletion) were observed in the bursa of the animals vaccinated with VP5⁺ recombinant IBDV vaccine or with Nobilis vaccine D78. In contrast animals not vaccinated and challenged showed severe lesions 10 days after challenge. In other words all animals (100%) of groups 1 and 3, vaccinated with the VP5⁻ recombinant IBDV vaccine or with Nobilis vaccine D78 were protected against severe challenge.

Three, 7, 14 and 20 days after vaccination and 3 and 10 days after challenge with the recombinant VP5⁻ IBDV vaccine, animals of group 2 showed no to hardly any lesions (0-20% lymphocytic depletion) in the bursa. All animals of group 2, vaccinated with the VP5⁻ recombinant IBDV vaccine, were protected against severe challenge. When animals vaccinated with the recombinant VP5⁻ IBDV vaccine are compared to animals of groups 1 or 3 (vaccinated with a recombinant VP5⁺ or commercial available vaccine) the recombinant VP5⁻ vaccine induces less lesions and therefore, is safer, milder than the vaccines tested in this experiment.

Three days post-challenge, all non-vaccinated animals of group 4 showed severe acute lesions in the bursa (total lymphocyte depletion, score 5.0). Ten days after challenge, all animals (17 out of 17 animals) showed total lymphocytic depletion, indicating that these animals were not protected against severe challenge. Animals that died after challenge, all showed severe lesions in the bursa of Fabricius. It was concluded that control group 4 was not protected against severe challenge indicating that the test conditions were optimal.

Table 4: Average bursal lesion score at different days after vaccination or challenge. The average lesion score is calculated as follows: all lesion scores from the animals per group on a certain day are added. This number is then divided by the total number of animals investigated in that group on that day. Individual scores range from 1 to 5. Score 0 = no lymphocytic depletion, score 1 = 0 - 20%; score 2 = 20 - 40%; score 3 = 40 - 60%; score 4 = 60 - 80% and score 5 = 80 - 100 % lymphocytic depletion (total lymphocytic depletion).

	Days after vaccination→				Days after challenge→		
	3	7	14	20	3	10	
Group↓	Bursal lesions score↓						Protection↓
1 VP5 ⁺	0.8	2.9	1.0	1.0	1.0 ^c	0.6	100%
2 VP5 ⁻	0.0	0.0	0.5	0.0	0.0 ^c	0.1	100%
3 D78	0.1	2.4	3.5	2.0	2.8 ^c	1.1	100%
4 -	0.0	0.0	0.0	0.0	5.0 ^a	5.0	0%

^a Acute lesions ^c Chronic lesions

Serological response.

The serological response of the animals was determined by measuring the ability of blood serum to neutralise a classical infectious bursal disease virus strain in a virus neutralising (VN) test. Serum was investigated 3, 7, 14 and 20 days after vaccination. The average neutralising titres are shown in Table 5.

The results show that recombinant IBDV vaccine VP5⁺ applied to chickens of group 1 induced a good and high serological response 20 days after vaccination which is comparable to the serological response of the chickens vaccinated with the commercial IBDV vaccine Nobilis strain D78 (group 3). The recombinant IBDV vaccine VP5⁻ applied to chickens of group 2 induced also a good serological response. A titre of 9.4 log₂ was observed 20 days after vaccination. The serological response induced by the recombinant VP5⁻ IBDV vaccine was delayed when compared to the serological response induced by the recombinant IBDV VP5⁺ vaccine or the commercial IBDV vaccine Nobilis strain D78.

The non-vaccinated group 4 showed no serological response to IBDV.

Table 5: Average IBDV-VN-titres for groups 1 to 4 at different days after vaccination, expressed as log₂ of the dilution.

Group	Days after vaccination			
	3	7	14	20
1 VP5 ⁺	$\leq 1.0 \pm 0.0$	7.1 ± 1.7	10.2 ± 1.4	11.9 ± 1.8
2 VP5 ⁻	$\leq 1.0 \pm 0.0$	2.1 ± 1.7	6.3 ± 2.9	9.4 ± 1.4
3 D78	$\leq 1.0 \pm 0.0$	5.2 ± 2.8	10.3 ± 1.3	11.6 ± 1.5
4 -	$\leq 1.0 \pm 0.0$	$\leq 1.0 \pm 0.0$	$\leq 1.0 \pm 0.0$	$\leq 1.0 \pm 0.0$

Serological differentiation between antisera.

The serological response against VP5 was investigated by making use of western blot analysis. For this purpose the VP5 protein was expressed in the E. coli or baculo expression system. The expressed proteins were separated by SDS PAGE. Next the proteins were electroblotted onto a nitro-cellulose membrane. Thereafter, the membrane was cut into lanes

and the lanes were incubated with rabbit anti-VP5 serum, chicken serum directed against VP5⁺ recombinant vaccine, chicken serum directed against VP5⁻ recombinant vaccine or negative serum from SPF chickens. Data are summarised in Table 6. As can be seen from Table 6, the VP5⁻ serum does not induce a serological response against VP5. In contrast the rabbit anti-VP5 serum and chicken serum directed against VP5⁺ recombinant vaccine do recognise the VP5-protein and thus induces a serological response against VP5. This indicates that chicken serum may be used to investigate if animals are exposed to a virus that expresses the VP5 protein (e.g. field virus) or to the VP5⁻ recombinant vaccine.

Table 6: Western blot analysis. Serum from animals vaccinated with VP5⁺ or VP5⁻ recombinant vaccine as well as SPF chicken serum and anti VP5-rabbit serum were investigated for their reaction with the VP5-protein.

Identification of serum sample	Immuno-blot
VP5 ⁺ vaccinated animal, serum sample 20d after vaccination	positive
VP5 ⁻ vaccinated animal, serum sample 20d after vaccination	negative
Non-vaccinated control, serum sample at 20d	negative
Rabbit anti VP5 serum	positive

Mortality and clinical signs.

None of the animals vaccinated with VP5⁺ IBDV vaccine (group 1), vaccinated with recombinant VP5⁻ IBDV vaccine (group 2) or vaccinated with the commercial IBDV vaccine Nobilis strain D78 (group 3), died or showed clinical signs of infectious bursal disease after challenge, indicating that the animals were protected against severe challenge. All animals in the non-vaccinated control group were not protected against severe challenge.

Example 4

In vivo testing of the recombinant VP5⁻-2 vaccine

Preparation of the IBDV vaccines. Primary chicken embryo fibroblasts (CEF) cells were prepared at a final concentration of 2×10^6 /ml. The cells were cultured in Eagles minimum essential medium containing 5% fetal calf serum. To 15 ml of this cell suspension 0.1 ml IBDV/VP5⁻-2 (D78/D78/VP5⁻) virus was added. After incubation for 6 days in a high humidity incubator at 37°C, the supernatant was titrated. The infectious titre of the supernatant was $10^{8.2}$ TCID₅₀/ml. For the second animal experiment the supernatant was diluted to result in a vaccine dose of $10^{5.5}$ TCID₅₀/animal and for the first animal experiment the supernatant was diluted to result in a vaccine dose of $10^{4.0}$ TCID₅₀/animal or $10^{5.0}$ TCID₅₀/egg.

First animal experiment. The effect of the vaccine is assessed by measurement of the serological response and resistance to challenge obtained from administering a challenge virus at the age of 14 days. The vaccine ($10^{5.0}$ TCID₅₀/egg or $10^{4.0}$ TCID₅₀/animal of D78/D78/VP5⁻) was applied *in ovo* or intramuscularly at day old. Microscopic lesions in the bursa were investigated, 3 and 10 days after challenge. Protection against challenge was determined and the serological response at the age of 14 days old was determined with the VN-test.

1. Average microscopic lesion score in the bursa 3 and 10 days after challenge.

Days post	Group		
challenge	<i>In ovo</i>	Day old	None-vaccinated
3	3.3	0.0	5.0
10	0.2	0.0	5.0

2. Protection after challenge

	Group		
	<i>In ovo</i>	Day old	None-vaccinated
% protection	91.6	100	0

3. Serological response against IBDV

	Group		
	<i>In ovo</i>	Day old	None-vaccinated
VN-titre	6.4 ± 1.7	6.4 ± 1.3	$<4.0 \pm 0.0$

VN-titre is expressed as log₂ of the dilution. Animals with a titre <4.0 log₂ are considered negative

5 Conclusions

- 1 The D78/D78/VP5⁻ strain is a highly attenuated IBD-virus
- 2 The virus strain is very mild
- 3 The virus can induce a serological response
- 4 The virus can induce protection
- 5 The virus strain can be applied by intramuscular injection to 1 day old SPF chickens and *in ovo* to 18-days-old embryonated SPF-eggs

Second animal experiment. The effect of the vaccine is assessed by measurement of the serological response against IBDV and resistance to challenge obtained from administering a challenge virus, 21 days after administering the Gumboro vaccine. The vaccine ($10^{5.5}$ TCID₅₀/animal of D78/D78/VP5⁻) was applied via the intramuscular route to 14 days old SPF-chickens. Three, 7, 14, and 20 days after vaccination and 3 days after challenge Bursa, spleen, thymus, liver, duodenum, pancreas, ceacal tonsils and harderian gland were investigated for microscopic lesions. Ten days after challenge Bursae were investigated for microscopic lesions. Sera were tested in the VN-test. And mortality was scored after challenge.

1. Percentage mortality after challenge:

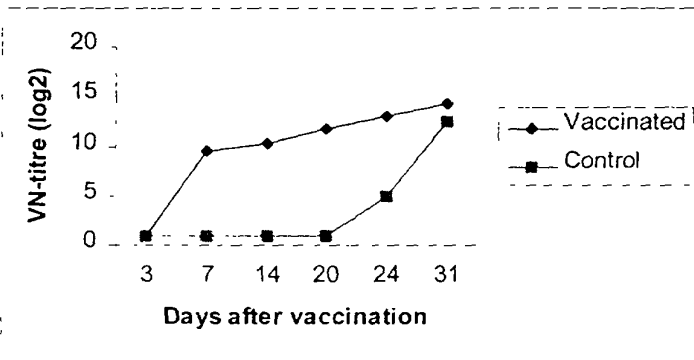
	Mortality after challenge
Vaccinated group	0%
Control group	50%

2. Microscopic lesions of the vaccinated group before and after challenge:

Days post	Bursa	Spleen	Thymus	Liver	Duodeum	Pancreas	Ceecal	Harderian
Vaccinat.							Tonsils	Gland
3	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
24	0,A	0	0	0	0	0	0	0
31	0,A	ND	ND	ND	ND	ND	ND	ND

A = None vaccinated animals showed a lymphocytic depletion score of 5.0 (100%) and 4.25, 3 and 10 days after challenge, respectively. ND = not done.

3. Serological response after vaccination:



Conclusions

1. The D78/D78/VP5⁻ strain is a highly attenuated IBD-virus
2. The virus strain is very mild and does not induce lesions in organs
3. The virus can induce a serological response
4. The virus can induce protection

LEGENDS TO THE FIGURES

Figure 1 Genomic organization of segment A and segment B of IBDV. The numbers indicate the nucleotide positions of the start, end and coding region on the segments.

Figure 2 Construction of genomic cDNA clones for the preparation of IBDV/VP5⁻. Plasmid pAD78/EK contains the complete D78 segment A cDNA encoding the polyprotein (VP2-VP4-VP3) and VP5. Plasmid pBP2 contains the complete strain P2 segment B encoding VP1. Mutations were introduced in plasmid pAD78/VP5⁻ altering the methionine start codon for VP5 into arginine and creating an artificial Afl II cleavage site. Recombinant plasmids were linearized with the underlined restriction enzymes, followed by T7 polymerase transcription.

Figure 3 Construction of genomic cDNA clones for the preparation of IBDV/VP5⁻-2. Plasmid pAD78/EK contains the complete D78 segment A cDNA encoding the polyprotein (VP2-VP4-VP3) and VP5. Plasmid pBD78 contains the complete strain D78 segment B encoding VP1. Mutations were introduced in plasmid pAD78/VP5⁻ altering the methionine start codon for VP5 into glutamic acid and creating an artificial BstBI cleavage site. Further mutations were introduced in the arginine and glutamine codon. Recombinant plasmids were linearized with the underlined restriction enzymes, followed by T7 polymerase transcription.

Figure 4 Radioimmunoprecipitation of proteins from CEC infected cells with recombinant IBDV. CEC infected cells with IBDV/VP5⁻ (lanes 1-3), IBDV/EK (lanes 4-6) and uninfected controls were immunoprecipitated with rabbit anti-IBDV serum (lanes 1, 4, 7), rabbit anti-VP5 serum (lanes 2, 5, 8) and mAb DIE 7 (lanes 3, 6, 9). Position of molecular mass markers (M) is indicated. Location of the viral proteins VP2, VP3, VP4 and VP5 are marked.

Figure 5 Replication kinetics of IBDV/EK and IBDV/VP5⁻. Infectious titers of supernatants (vertical axis) are determined at the times indicated.

SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT:

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(ii) TITLE OF INVENTION: Recombinant birnavirus vaccine

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
20 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 2827 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- 40 (A) NAME/KEY: CDS
(B) LOCATION: 112..2745

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

45 GGATACGATG GGTCTGACCC TCTGGGAGTC ACGAATTAAC GTGGCTACTA GGGGCGATAC

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	Met Ser	
	1	
5	GAC ATT TTC AAC AGT CCA CAG GCG CGA AGC ACG ATC TCA GCA GCG TTC	165
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	5 10 15	
10	GGC ATA AAG CCT ACT GCT GGA CAA GAC GTG GAA GAA CTC TTG ATC CCT	213
	Gly Ile Lys Pro Thr Ala Gly Gln Asp Val Glu Glu Leu Leu Ile Pro	
	20 25 30	
15	AAA GTT TGG GTG CCA CCT GAG GAT CCG CTT GCC AGC CCT AGT CGA CTG	261
	Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser Arg Leu	
	35 40 45 50	
20	GCA AAG TTC CTC AGA GAG AAC GGC TAC AAA GTT TTG CAG CCA CGG TCT	309
	Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Val Leu Gln Pro Arg Ser	
	55 60 65	
25	CTG CCC GAG AAT GAG GAG TAT GAG ACC GAC CAA ATA CTC CCA GAC TTA	357
	Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro Asp Leu	
	70 75 80	
30	GCA TGG ATG CGA CAG ATA GAA GGG GCT GTT TTA AAA CCC ACT CTA TCT	405
	Ala Trp Met Arg Gln Ile Glu Gly Ala Val Leu Lys Pro Thr Leu Ser	
	85 90 95	
35	CTC CCT ATT GGA GAT CAG GAG TAC TTC CCA AAG TAC TAC CCA ACA CAT	453
	Leu Pro Ile Gly Asp Gln Glu Tyr Phe Pro Lys Tyr Tyr Pro Thr His	
	100 105 110	
40	CGC CCT AGC AAG GAG AAG CCC AAT GCG TAC CCG CCA GAC ATC GCA CTA	501
	Arg Pro Ser Lys Glu Lys Pro Asn Ala Tyr Pro Pro Asp Ile Ala Leu	
	115 120 125 130	
45	CTC AAG CAG ATG ATT TAC CTG TTT CTC CAG GTT CCA GAG GCC AAC GAG	549
	Leu Lys Gln Met Ile Tyr Leu Phe Leu Gln Val Pro Glu Ala Asn Glu	
	135 140 145	
50	GGC CTA AAG GAT GAA GTA ACC CTC TTG ACC CAA AAC ATA AGG GAC AAG	597
	Gly Leu Lys Asp Glu Val Thr Leu Leu Thr Gln Asn Ile Arg Asp Lys	
	150 155 160	
55	GCC TAT GGA AGT GGG ACC TAC ATG GGA CAA GCA AAT CGA CTT GTG GCC	645
	Ala Tyr Gly Ser Gly Thr Tyr Met Gly Gln Ala Asn Arg Leu Val Ala	
	165 170 175	

	ATG AAG GAG GTC GCC ACT GGA AGA AAC CCA AAC AAG GAT CCT CTA AAG	693
	Met Lys Glu Val Ala Thr Gly Arg Asn Pro Asn Lys Asp Pro Leu Lys	
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5	CTT GGG TAC ACT TTT GAG AGC ATC GCG CAG CTA CTT GAC ATC ACA CTA	741
	Leu Gly Tyr Thr Phe Glu Ser Ile Ala Gln Leu Leu Asp Ile Thr Leu	
	195 200 205 210	
10	CCG GTA GGC CCA CCC GGT GAG GAT GAC AAG CCC TGG GTG CCA CTC ACA	789
	Pro Val Gly Pro Pro Gly Glu Asp Asp Lys Pro Trp Val Pro Leu Thr	
	215 220 225	
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	Arg Val Pro Ser Arg Met Leu Val Leu Thr Gly Asp Val Asp Gly Asp	
	230 235 240	
20	TTT GAG GTT GAA GAT TAC CTT CCC AAA ATC AAC CTC AAG TCA TCA AGT	885
	Phe Glu Val Glu Asp Tyr Leu Pro Lys Ile Asn Leu Lys Ser Ser Ser	
	245 250 255	
25	GGA CTA CCA TAT GTA GGT CGC ACC AAA GGA GAG ACA ATT GGC GAG ATG	933
	Gly Leu Pro Tyr Val Gly Arg Thr Lys Gly Glu Thr Ile Gly Glu Met	
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30	ATA GCT ATC TCA AAC CAG TTT CTC AGA GAG CTA TCA ACA CTG TTG AAG	981
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	295 300 305	
40	TTA AGT GAC TAT TGG TAC TTA TCA TGC GGG CTT TTG TTT CCA AAG GCT	1077
	Leu Ser Asp Tyr Trp Tyr Leu Ser Cys Gly Leu Leu Phe Pro Lys Ala	
	310 315 320	
45	GAA AGG TAC GAC AAA AGT ACA TGG CTC ACC AAG ACC CGG AAC ATA TGG	1125
	Glu Arg Tyr Asp Lys Ser Thr Trp Leu Thr Lys Thr Arg Asn Ile Trp	
	325 330 335	
50	TCA GCT CCA TCC CCA ACA CAC CTC ATG ATC TCT ATG ATC ACC TGG CCC	1173
	Ser Ala Pro Ser Pro Thr His Leu Met Ile Ser Met Ile Thr Trp Pro	
	340 345 350	
55	GTG ATG TCC AAC AGC CCA AAT AAC GTG TTG AAC ATT GAA GGG TGT CCA	1221
	Val Met Ser Asn Ser Pro Asn Asn Val Leu Asn Ile Glu Gly Cys Pro	
	355 360 365 370	

	TCA CTC TAC AAA TTC AAC CCG TTC AGA GGA GGG TTG AAC AGG ATC GTC	1269
	Ser Leu Tyr Lys Phe Asn Pro Phe Arg Gly Gly Leu Asn Arg Ile Val	
	375 380 385	
5	GAG TGG ATA TTG GCC CCG GAA GAA CCC AAG GCT CTT GTA TAT GCG GAC	1317
	Glu Trp Ile Leu Ala Pro Glu Glu Pro Lys Ala Leu Val Tyr Ala Asp	
	390 395 400	
10	AAC ATA TAC ATT GTC CAC TCA AAC ACG TGG TAC TCA ATT GAC CTA GAG	1365
	Asn Ile Tyr Ile Val His Ser Asn Thr Trp Tyr Ser Ile Asp Leu Glu	
	405 410 415	
15	AAG GGT GAG GCA AAC TGC ACT CGC CAA CAC ATG CAA GCC GCA ATG TAC	1413
	Lys Gly Glu Ala Asn Cys Thr Arg Gln His Met Gln Ala Ala Met Tyr	
	420 425 430	
20	TAC ATA CTC ACC AGA GGG TGG TCA GAC AAC GGC GAC CCA ATG TTC AAT	1461
	Tyr Ile Leu Thr Arg Gly Trp Ser Asp Asn Gly Asp Pro Met Phe Asn	
	435 440 445 450	
25	CAA ACA TGG GCC ACC TTT GCC ATG AAC ATT GCC CCT GCT CTA GTG GTG	1509
	Gln Thr Trp Ala Thr Phe Ala Met Asn Ile Ala Pro Ala Leu Val Val	
	455 460 465	
30	GAC TCA TCG TGC CTG ATA ATG AAC CTG CAA ATT AAG ACC TAT GGT CAA	1557
	Asp Ser Ser Cys Leu Ile Met Asn Leu Gln Ile Lys Thr Tyr Gly Gln	
	470 475 480	
35	GGC AGC GGG AAT GCA GCC ACG TTC ATC AAC CAC CTC TTG AGC ACA	1605
	Gly Ser Gly Asn Ala Ala Thr Phe Ile Asn Asn His Leu Leu Ser Thr	
	485 490 495	
40	CTA GTG CTT GAC CAG TGG AAC CTG ATG AGA CAG CCC AGA CCA GAC AGC	1653
	Leu Val Leu Asp Gln Trp Asn Leu Met Arg Gln Pro Arg Pro Asp Ser	
	500 505 510	
45	GAG GAG TTC AAA TCA ATT GAG GAC AAG CTA GGT ATC AAC TTT AAG ATT	1701
	Glu Glu Phe Lys Ser Ile Glu Asp Lys Leu Gly Ile Asn Phe Lys Ile	
	515 520 525 530	
50	GAG AGG TCC ATT GAT GAT ATC AGG GGC AAG CTG AGA CAG CTT GTC CTC	1749
	Glu Arg Ser Ile Asp Asp Ile Arg Gly Lys Leu Arg Gln Leu Val Leu	
	535 540 545	
55	CTT GCA CAA CCA GGG TAC CTG AGT GGG GGG GTT GAA CCA GAA CAA TCC	1797
	Leu Ala Gln Pro Gly Tyr Leu Ser Gly Gly Val Glu Pro Glu Gln Ser	
	550 555 560	

	AGC CCA ACT GTT GAG CTT GAC CTA CTA GGG TGG TCA GCT ACA TAC AGC	1845
	Ser Pro Thr Val Glu Leu Asp Leu Leu Gly Trp Ser Ala Thr Tyr Ser	
	565 570 575	
5	AAA GAT CTC GGG ATC TAT GTG CCG GTG CTT GAC AAG GAA CGC CTA TTT	1893
	Lys Asp Leu Gly Ile Tyr Val Pro Val Leu Asp Lys Glu Arg Leu Phe	
	580 585 590	
10	TGT TCT GCT GCG TAT CCC AAG GGA GTA GAG AAC AAG AGT CTC AAG TCC	1941
	Cys Ser Ala Ala Tyr Pro Lys Gly Val Glu Asn Lys Ser Leu Lys Ser	
	595 600 605 610	
15	AAA GTC GGG ATC GAG CAG GCA TAC AAG GTA GTC AGG TAT GAG GCG TTG	1989
	Lys Val Gly Ile Glu Gln Ala Tyr Lys Val Val Arg Tyr Glu Ala Leu	
	615 620 625	
20	AGG TTG GTA GGT GGT TGG AAC TAC CCA CTC CTG AAC AAA GCC TGC AAG	2037
	Arg Leu Val Gly Gly Trp Asn Tyr Pro Leu Leu Asn Lys Ala Cys Lys	
	630 635 640	
25	AAT AAC GCA GGC GCC GCT CGG CGG CAT CTG GAG GCC AAG GGG TTC CCA	2085
	Asn Asn Ala Gly Ala Ala Arg Arg His Leu Glu Ala Lys Gly Phe Pro	
	645 650 655	
30	CTC GAC GAG TTC CTA GCC GAG TGG TCT GAG CTG TCA GAG TTC GGT GAG	2133
	Leu Asp Glu Phe Leu Ala Glu Trp Ser Glu Leu Ser Glu Phe Gly Glu	
	660 665 670	
35	GCC TTC GAA GGC TTC AAT ATC AAG CTG ACC GTA ACA TCT GAG AGC CTA	2181
	Ala Phe Glu Gly Phe Asn Ile Lys Leu Thr Val Thr Ser Glu Ser Leu	
	675 680 685 690	
40	GCC GAA CTG AAC AAG CCA GTA CCC CCC AAG CCC CCA AAT GTC AAC AGA	2229
	Ala Glu Leu Asn Lys Pro Val Pro Pro Lys Pro Pro Asn Val Asn Arg	
	695 700 705	
45	CCA GTC AAC ACT GGG GGA CTC AAG GCA GTC AGC AAC GCC CTC AAG ACC	2277
	Pro Val Asn Thr Gly Gly Leu Lys Ala Val Ser Asn Ala Leu Lys Thr	
	710 715 720	
50	GGT CGG TAC AGG AAC GAA GCC GGA CTG AGT GGT CTC GTC CTT CTA GCC	2325
	Gly Arg Tyr Arg Asn Glu Ala Gly Leu Ser Gly Leu Val Leu Leu Ala	
	725 730 735	
55	ACA GCA AGA AGC CGT CTG CAA GAT GCA GTT AAG GCC AAG GCA GAA GCC	2373
	Thr Ala Arg Ser Arg Leu Gln Asp Ala Val Lys Ala Lys Ala Glu Ala	
	740 745 750	

	GAG AAA CTC CAC AAG TCC AAG CCA GAC GAC CCC GAT GCA GAC TGG TTC	2421
	Glu Lys Leu His Lys Ser Lys Pro Asp Asp Pro Asp Ala Asp Trp Phe	
	755 760 765 770	
5	GAA AGA TCA GAA ACT CTG TCA GAC CTT CTG GAG AAA GCC GAC ATC GCC	2469
	Glu Arg Ser Glu Thr Leu Ser Asp Leu Leu Glu Lys Ala Asp Ile Ala	
	775 780 785	
10	AGC AAG GTC GCC CAC TCA GCA CTC GTG GAA ACA AGC GAC GCC CTT GAA	2517
	Ser Lys Val Ala His Ser Ala Leu Val Glu Thr Ser Asp Ala Leu Glu	
	790 795 800	
15	GCA GTT CAG TCG ACT TCC GTG TAC ACC CCC AAG TAC CCA GAA GTC AAG	2565
	Ala Val Gln Ser Thr Ser Val Tyr Thr Pro Lys Tyr Pro Glu Val Lys	
	805 810 815	
20	AAC CCA CAG ACC GCC TCC AAC CCC GTT GTT GGG CTC CAC CTG CCC GCC	2613
	Asn Pro Gln Thr Ala Ser Asn Pro Val Val Gly Leu His Leu Pro Ala	
	820 825 830	
25	AAG AGA GCC ACC GGT GTC CAG GCC GCT CTT CTC GGA GCA GGA ACG AGC	2661
	Lys Arg Ala Thr Gly Val Gln Ala Ala Leu Leu Gly Ala Gly Thr Ser	
	835 840 845 850	
30	AGA CCA ATG GGG ATG GAG GCC CCA ACA CGG TCC AAG AAC GCC GTG AAA	2709
	Arg Pro Met Gly Met Glu Ala Pro Thr Arg Ser Lys Asn Ala Val Lys	
	855 860 865	
35	ATG GCC AAA CGG CGG CAA CGC CAA AAG GAG ACC CGC TAACAGCCAT	2755
	Met Ala Lys Arg Arg Gln Arg Gln Lys Glu Ser Arg	
	870 875	
	GATGGGAACC ACTCAAGAAG AGGACACTAA TCCCAGACCC CGTATCCCCG GCCTTCGCCT	2815
	GCGGGGGCCC CC	2827

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 878 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Asp Ile Phe Asn Ser Pro Gln Ala Arg Ser Thr Ile Ser Ala
 1 5 10 15
 Ala Phe Gly Ile Lys Pro Thr Ala Gly Gln Asp Val Glu Glu Leu Leu
 5 20 25 30
 Ile Pro Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser
 35 40 45
 Arg Leu Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Val Leu Gln Pro
 50 55 60
 Arg Ser Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro
 65 70 75 80
 Asp Leu Ala Trp Met Arg Gln Ile Glu Gly Ala Val Leu Lys Pro Thr
 85 90 95
 Leu Ser Leu Pro Ile Gly Asp Gln Glu Tyr Phe Pro Lys Tyr Tyr Pro
 100 105 110
 Thr His Arg Pro Ser Lys Glu Lys Pro Asn Ala Tyr Pro Pro Asp Ile
 115 120 125
 Ala Leu Leu Lys Gln Met Ile Tyr Leu Phe Leu Gln Val Pro Glu Ala
 130 135 140
 Asn Glu Gly Leu Lys Asp Glu Val Thr Leu Leu Thr Gln Asn Ile Arg
 145 150 155 160
 Asp Lys Ala Tyr Gly Ser Gly Thr Tyr Met Gly Gln Ala Asn Arg Leu
 165 170 175
 Val Ala Met Lys Glu Val Ala Thr Gly Arg Asn Pro Asn Lys Asp Pro
 180 185 190
 Leu Lys Leu Gly Tyr Thr Phe Glu Ser Ile Ala Gln Leu Leu Asp Ile
 195 200 205
 Thr Leu Pro Val Gly Pro Pro Gly Glu Asp Asp Lys Pro Trp Val Pro
 210 215 220
 Leu Thr Arg Val Pro Ser Arg Met Leu Val Leu Thr Gly Asp Val Asp
 225 230 235 240
 Gly Asp Phe Glu Val Glu Asp Tyr Leu Pro Lys Ile Asn Leu Lys Ser
 245 250 255

Ser Ser Gly Leu Pro Tyr Val Gly Arg Thr Lys Gly Glu Thr Ile Gly
 260 265 270
 5 Glu Met Ile Ala Ile Ser Asn Gln Phe Leu Arg Glu Leu Ser Thr Leu
 275 280 285
 Leu Lys Gln Gly Ala Gly Thr Lys Gly Ser Asn Lys Lys Lys Leu Leu
 290 295 300
 10 Ser Met Leu Ser Asp Tyr Trp Tyr Leu Ser Cys Gly Leu Leu Phe Pro
 305 310 315 320
 Lys Ala Glu Arg Tyr Asp Lys Ser Thr Trp Leu Thr Lys Thr Arg Asn
 325 330 335
 15 Ile Trp Ser Ala Pro Ser Pro Thr His Leu Met Ile Ser Met Ile Thr
 340 345 350
 20 Trp Pro Val Met Ser Asn Ser Pro Asn Asn Val Leu Asn Ile Glu Gly
 355 360 365
 Cys Pro Ser Leu Tyr Lys Phe Asn Pro Phe Arg Gly Gly Leu Asn Arg
 370 375 380
 25 Ile Val Glu Trp Ile Leu Ala Pro Glu Glu Pro Lys Ala Leu Val Tyr
 385 390 395 400
 Ala Asp Asn Ile Tyr Ile Val His Ser Asn Thr Trp Tyr Ser Ile Asp
 405 410 415
 30 Leu Glu Lys Gly Glu Ala Asn Cys Thr Arg Gln His Met Gln Ala Ala
 420 425 430
 35 Met Tyr Tyr Ile Leu Thr Arg Gly Trp Ser Asp Asn Gly Asp Pro Met
 435 440 445
 Phe Asn Gln Thr Trp Ala Thr Phe Ala Met Asn Ile Ala Pro Ala Leu
 450 455 460
 40 Val Val Asp Ser Ser Cys Leu Ile Met Asn Leu Gln Ile Lys Thr Tyr
 465 470 475 480
 Gly Gln Gly Ser Gly Asn Ala Ala Thr Phe Ile Asn Asn His Leu Leu
 485 490 495
 45 Ser Thr Leu Val Leu Asp Gln Trp Asn Leu Met Arg Gln Pro Arg Pro
 500 505 510

Asp Ser Glu Glu Phe Lys Ser Ile Glu Asp Lys Leu Gly Ile Asn Phe
 515 520 525
 Lys Ile Glu Arg Ser Ile Asp Asp Ile Arg Gly Lys Leu Arg Gln Leu
 5 530 535 540
 Val Leu Leu Ala Gln Pro Gly Tyr Leu Ser Gly Gly Val Glu Pro Glu
 545 550 555 560
 Gln Ser Ser Pro Thr Val Glu Leu Asp Leu Leu Gly Trp Ser Ala Thr
 10 565 570 575
 Tyr Ser Lys Asp Leu Gly Ile Tyr Val Pro Val Leu Asp Lys Glu Arg
 580 585 590
 Leu Phe Cys Ser Ala Ala Tyr Pro Lys Gly Val Glu Asn Lys Ser Leu
 595 600 605
 Lys Ser Lys Val Gly Ile Glu Gln Ala Tyr Lys Val Val Arg Tyr Glu
 20 610 615 620
 Ala Leu Arg Leu Val Gly Gly Trp Asn Tyr Pro Leu Leu Asn Lys Ala
 625 630 635 640
 Cys Lys Asn Asn Ala Gly Ala Ala Arg Arg His Leu Glu Ala Lys Gly
 25 645 650 655
 Phe Pro Leu Asp Glu Phe Leu Ala Glu Trp Ser Glu Leu Ser Glu Phe
 660 665 670
 Gly Glu Ala Phe Glu Gly Phe Asn Ile Lys Leu Thr Val Thr Ser Glu
 30 675 680 685
 Ser Leu Ala Glu Leu Asn Lys Pro Val Pro Pro Lys Pro Pro Asn Val
 35 690 695 700
 Asn Arg Pro Val Asn Thr Gly Gly Leu Lys Ala Val Ser Asn Ala Leu
 705 710 715 720
 Lys Thr Gly Arg Tyr Arg Asn Glu Ala Gly Leu Ser Gly Leu Val Leu
 40 725 730 735
 Leu Ala Thr Ala Arg Ser Arg Leu Gln Asp Ala Val Lys Ala Lys Ala
 740 745 750
 Glu Ala Glu Lys Leu His Lys Ser Lys Pro Asp Asp Pro Asp Ala Asp
 45 755 760 765

Trp Phe Glu Arg Ser Glu Thr Leu Ser Asp Leu Leu Glu Lys Ala Asp
 770 775 780

Ile Ala Ser Lys Val Ala His Ser Ala Leu Val Glu Thr Ser Asp Ala
 5 785 790 795 800

Leu Glu Ala Val Gln Ser Thr Ser Val Tyr Thr Pro Lys Tyr Pro Glu
 805 810 815

10 Val Lys Asn Pro Gln Thr Ala Ser Asn Pro Val Val Gly Leu His Leu
 820 825 830

Pro Ala Lys Arg Ala Thr Gly Val Gln Ala Ala Leu Leu Gly Ala Gly
 835 840 845

15 Thr Ser Arg Pro Met Gly Met Glu Ala Pro Thr Arg Ser Lys Asn Ala
 850 855 860

20 Val Lys Met Ala Lys Arg Arg Gln Arg Gln Lys Glu Ser Arg
 865 870 875

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3261 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 97..531

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGATACGATC GGTCTGACCC CGGGGGAGTC ACCCGGGGAC AGGCCGTCAA GGCCTTGTTT 60

45 CAGGATGGGA CTCCTCCTTC TACAACGCTA TCATTG ATG GTT AGT AGA GAT CAG 114
 Met Val Ser Arg Asp Gln

	ACA AAC GAT CGC AGC GAT GAC AAA CCT GCA AGA TCA AAC CCA ACA GAT	162
	Thr Asn Asp Arg Ser Asp Asp Lys Pro Ala Arg Ser Asn Pro Thr Asp	
	10 15 20	
5	TGT TCC GTT CAT ACG GAG CCT TCT GAT GCC AAC AAC CGG ACC GGC GTC	210
	Cys Ser Val His Thr Glu Pro Ser Asp Ala Asn Asn Arg Thr Gly Val	
	25 30 35	
	CAT TCC GGA CGA CAC CCT GGA GAA GCA CAC TCT CAG GTC AGA GAC CTC	258
10	His Ser Gly Arg His Pro Gly Glu Ala His Ser Gln Val Arg Asp Leu	
	40 45 50	
	GAC CTA CAA TTT GAC TGT GGG GGA CAC AGG GTC AGG GCT AAT TGT CTT	306
15	Asp Leu Gln Phe Asp Cys Gly Gly His Arg Val Arg Ala Asn Cys Leu	
	55 60 65 70	
	TTT CCC TGG ATT CCC TGG CTC AAT TGT GGG TGC TCA CTA CAC ACT GCA	354
	Phe Pro Trp Ile Pro Trp Leu Asn Cys Gly Cys Ser Leu His Thr Ala	
	75 80 85	
20	GGG CAA TGG GAA CTA CAA GTT CGA TCA GAT GCT CCT GAC TGC CCA GAA	402
	Gly Gln Trp Glu Leu Gln Val Arg Ser Asp Ala Pro Asp Cys Pro Glu	
	90 95 100	
25	CCT ACC GGC CAG TTA CAA CTA CTG CAG GCT AGT GAG TCG GAG TCT CAC	450
	Pro Thr Gly Gln Leu Gln Leu Leu Gln Ala Ser Glu Ser Glu Ser His	
	105 110 115	
	AGT GAG GTC AAG CAC ACT TCC TGG TGG CGT TTT TGC ACT AAA CGG CAC	498
30	Ser Glu Val Lys His Thr Ser Trp Trp Arg Leu Cys Thr Lys Arg His	
	120 125 130	
	CAT AAA CGC CGT GAC CTT CCA AGG AAG CCT GAG TGAAGTACACA GATGTTAGCT	551
35	His Lys Arg Arg Asp Leu Pro Arg Lys Pro Glu	
	135 140 145	
	ACAATGGGTT GATGTCTGCA ACAGCCAACA TCAACGACAA AATTGGGAAC GTCCTAGTAG	611
	GGGAAGGGGT CACCGTCCTC AGCTTACCCA CATCATATGA TCTTGGGTAT GTGAGGCTTG	671
40	GTGACCCCAT TCCC GCAATA GGGCTTGACC CAAAAATGGT AGCCACATGT GACAGCAGTG	731
	ACAGGCCCAG AGTCTACACC ATAAGTGCAG CCGATGATTA CCAATTCTCA TCACAGTACC	791
45	AACCAGGTGG GGTAACAATC AACTGTTCT CAGCCAACAT TGATGCCATC ACAAGCCTCA	851
	GCGTTGGGGG AGAGCTCGTG TTTCAAACAA GCGTCCACGG CTTGTACTG GGCGCCACCA	911

	TCTACCTCAT	AGGCTTTGAT	GGGACAACGG	TAATCACCAG	GGCTGTGGCC	GCAAACAATG	971
	GGCTGACGAC	CGGCACCGAC	AACCTTATGC	CATTCAATCT	TGTGATTCCA	ACAAACGAGA	1031
5	TAACCCAGCC	AATCACATCC	ATCAAACCTGG	AGATAGTGAC	CTCCAAAAGT	GGTGGTCAGG	1091
	CAGGGGATCA	GATGTCATGG	TCGGCAAGAG	GGAGCCTAGC	AGTGACGATC	CATGGTGGCA	1151
	ACTATCCAGG	GGCCCTCCGT	CCCGTCACGC	TAGTGGCCTA	CGAAAGAGTG	GCAACAGGAT	1211
10	CCGTCGTTAC	GGTCGCTGGG	GTGAGCAACT	TCGAGCTGAT	CCCAAATCCT	GAAGTAGCAA	1271
	AGAACCTGGT	TACAGAATAC	GGCCGATTTG	ACCCAGGAGC	CATGAACTAC	ACAAAATTGA	1331
15	TACTGAGTGA	GAGGGACCGT	CTTGGCATCA	AGACCGTCTG	GCCAACAAGG	GAGTACACTG	1391
	ACTTTCGTGA	ATACTTCATG	GAGGTGGCCG	ACCTCAACTC	TCCCCTGAAG	ATTGCAGGAG	1451
	CATTGCGCTT	CAAAGACATA	ATCCGGGCCA	TAAGGAGGAT	AGCTGTGCCG	GTGGTCTCCA	1511
20	CATTGTTCCC	ACCTGCCGCT	CCCCTAGCCC	ATGCAATTGG	GGAAGGTGTA	GACTACCTGC	1571
	TGGGCGATGA	GGCACAGGCT	GCTTCAGGAA	CTGCTCGAGC	CGCGTCAGGA	AAAGCAAGAG	1631
25	CTGCCTCAGG	CCGCATAAGG	CAGCTGACTC	TCGCCGCCGA	CAAGGGGTAC	GAGGTAGTCG	1691
	CGAATCTATT	CCAGGTGCCC	CAGAATCCCC	TAGTCGACGG	GATTCTTGCT	TCACCTGGGG	1751
	TACTCCGCGG	TGCACACAAC	CTCGACTGCG	TGTTTAGAGA	GGGTGCCACG	CTATTCCCTG	1811
30	TGGTTATTAC	GACAGTGGAA	GACGCCATGA	CACCCAAAGC	ATTGAACAGC	AAAATGTTTG	1871
	CTGTCATTGA	AGGCGTGCGA	GAAGACCTCC	AACCTCCATC	TCAAAGAGGA	TCCTTCATAC	1931
35	GAAGTCTCTC	TGGACACAGA	GTCTATGGAT	ATGCTCCAGA	TGGGGTACTT	CCACTGGAGA	1991
	CTGGGAGAGA	CTACACCGTT	GTCCCAATAG	ATGATGTCTG	GGACGACAGC	ATTATGCTGT	2051
	CCAAAGATCC	CATACCTCCT	ATTGTGGGAA	ACAGTGGAAA	TCTAGCCATA	GCTTACATGG	2111
40	ATGTGTTTCG	ACCCAAAGTC	CCAATCCATG	TGGCTATGAC	GGGAGCCCTC	AATGCTTGTG	2171
	GCGAGATTGA	GAAAGTAAGC	TTTAGAAGCA	CCAAGCTCGC	CACTGCACAC	CGACTTGGCC	2231
45	TTAGGTTGGC	TGGTCCCGGA	GCATTGATG	TAAACACCGG	GCCCAACTGG	GCAACGTTCA	2291
	TCAAACGTTT	CCCTCACAAAT	CCACGCGACT	GGGACAGGCT	CCCCTACCTC	AACCTACCAT	2351

ACCTTCCACC CAATGCAGGA CGCCAGTACC ACCTTGCCAT GGCTGCATCA GAGTTCAAAG 2411
 AGACCCCCGA ACTCGAGAGT GCCGTCAGAG CAATGGAAGC AGCAGCCAAC GTGGACCCAC 2471
 5 TATTCCAATC TGCACTCAGT GTGTTTCATGT GGCTGGAAGA GAATGGGATT GTGACTGACA 2531
 TGGCCAACTT CGCACTCAGC GACCCGAACG CCCATCGGAT GCGAAATTTT CTTGCAAACG 2591
 CACCACAAGC AGGCAGCAAG TCGCAAAGGG CCAAGTACGG GACAGCAGGC TACGGAGTGG 2651
 10 AGGCTCGGGG CCCACACCA GAGGAAGCAC AGAGGGAAAA AGACACACGG ATCTCAAAGA 2711
 AGATGGAGAC CATGGGCATC TACTTTGCAA CACCAGAATG GGTAGCACTC AATGGGCACC 2771
 15 GAGGGCCAAG CCCCGGCCAG CTAAAGTACT GGCAGAACAC ACGAGAAATA CCGGACCCAA 2831
 ACGAGGACTA TCTAGACTAC GTGCATGCAG AGAAGAGCCG GTTGGCATCA GAAGAACAAA 2891
 TCCTAAGGGC AGCTACGTCG ATCTACGGGG CTCCAGGACA GGCAGAGCCA CCCCAAGCTT 2951
 20 TCATAGACGA AGTTGCCAAA GTCTATGAAA TCAACCATGG ACGTGGCCCA AACCAAGAAC 3011
 AGATGAAAGA TCTGCTCTTG ACTGCGATGG AGATGAAGCA TCGCAATCCC AGGCGGGGCTC 3071
 25 TACCAAAGCC CAAGCCAAAA CCCAATGCTC CAACACAGAG ACCCCCTGGT CGGCTGGGCC 3131
 GCTGGATCAG GACCGTCTCT GATGAGGACC TTGAGTGAGG CTCCTGGGAG TCTCCCGACA 3191
 CCACCCGCGC AGGTGTGGAC ACCAATTCGG CTTACAACA TCCCAAATTG GATCCGTTTCG 3251
 30 CGGGTCCCCT 3261

35 (2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 145 amino acids

(B) TYPE: amino acid

40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

45 Met Val Ser Arg Asp Gln Thr Asn Asp Arg Ser Asp Asp Lys Pro Ala
 1 5 10 15

Arg Ser Asn Pro Thr Asp Cys Ser Val His Thr Glu Pro Ser Asp Ala
 20 25 30

5 Asn Asn Arg Thr Gly Val His Ser Gly Arg His Pro Gly Glu Ala His
 35 40 45

Ser Gln Val Arg Asp Leu Asp Leu Gln Phe Asp Cys Gly Gly His Arg
 50 55 60

10 Val Arg Ala Asn Cys Leu Phe Pro Trp Ile Pro Trp Leu Asn Cys Gly
 65 70 75 80

Cys Ser Leu His Thr Ala Gly Gln Trp Glu Leu Gln Val Arg Ser Asp
 85 90 95

15 Ala Pro Asp Cys Pro Glu Pro Thr Gly Gln Leu Gln Leu Leu Gln Ala
 100 105 110

20 Ser Glu Ser Glu Ser His Ser Glu Val Lys His Thr Ser Trp Trp Arg
 115 120 125

Leu Cys Thr Lys Arg His His Lys Arg Arg Asp Leu Pro Arg Lys Pro
 130 135 140

25 Glu
 145

30 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3261 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:131..3166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGATACGATC GGTCTGACCC CGGGGGAGTC ACCCGGGGAC AGGCCGTCAA GGCCTTGTTT

	CAGGATGGGA CTCCTCCTTC TACAACGCTA TCATTGATGG TTAGTAGAGA TCAGACAAAC	120
5	GATCGCAGCG ATG ACA AAC CTG CAA GAT CAA ACC CAA CAG ATT GTT CCG Met Thr Asn Leu Gln Asp Gln Thr Gln Gln Ile Val Pro 1 5 10	169
10	TTC ATA CGG AGC CTT CTG ATG CCA ACA ACC GGA CCG GCG TCC ATT CCG Phe Ile Arg Ser Leu Leu Met Pro Thr Thr Gly Pro Ala Ser Ile Pro 15 20 25	217
15	GAC GAC ACC CTG GAG AAG CAC ACT CTC AGG TCA GAG ACC TCG ACC TAC Asp Asp Thr Leu Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr 30 35 40 45	265
20	AAT TTG ACT GTG GGG GAC ACA GGG TCA GGG CTA ATT GTC TTT TTC CCT Asn Leu Thr Val Gly Asp Thr Gly Ser Gly Leu Ile Val Phe Phe Pro 50 55 60	313
25	GGA TTC CCT GGC TCA ATT GTG GGT GCT CAC TAC ACA CTG CAG GGC AAT Gly Phe Pro Gly Ser Ile Val Gly Ala His Tyr Thr Leu Gln Gly Asn 65 70 75	361
30	GGG AAC TAC AAG TTC GAT CAG ATG CTC CTG ACT GCC CAG AAC CTA CCG Gly Asn Tyr Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro 80 85 90	409
35	GCC AGT TAC AAC TAC TGC AGG CTA GTG AGT CGG AGT CTC ACA GTG AGG Ala Ser Tyr Asn Tyr Cys Arg Leu Val Ser Arg Ser Leu Thr Val Arg 95 100 105	457
40	TCA AGC ACA CTT CCT GGT GGC GTT TAT GCA CTA AAC GGC ACC ATA AAC Ser Ser Thr Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr Ile Asn 110 115 120 125	505
45	GCC GTG ACC TTC CAA GGA AGC CTG AGT GAA CTG ACA GAT GTT AGC TAC Ala Val Thr Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr 130 135 140	553
	AAT GGG TTG ATG TCT GCA ACA GCC AAC ATC AAC GAC AAA ATT GGG AAC Asn Gly Leu Met Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn 145 150 155	601
	GTC CTA GTA GGG GAA GGG GTC ACC GTC CTC AGC TTA CCC ACA TCA TAT Val Leu Val Gly Glu Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr 160 165 170	649
	GAT CTT GGG TAT GTG AGG CTT GGT GAC CCC ATT CCC GCA ATA GGG CTT	697

	Asp	Leu	Gly	Tyr	Val	Arg	Leu	Gly	Asp	Pro	Ile	Pro	Ala	Ile	Gly	Leu	
	175						180					185					
5	GAC	CCA	AAA	ATG	GTA	GCC	ACA	TGT	GAC	AGC	AGT	GAC	AGG	CCC	AGA	GTC	745
	Asp	Pro	Lys	Met	Val	Ala	Thr	Cys	Asp	Ser	Ser	Asp	Arg	Pro	Arg	Val	
	190					195				200					205		
10	TAC	ACC	ATA	ACT	GCA	GCC	GAT	GAT	TAC	CAA	TTC	TCA	TCA	CAG	TAC	CAA	793
	Tyr	Thr	Ile	Thr	Ala	Ala	Asp	Asp	Tyr	Gln	Phe	Ser	Ser	Gln	Tyr	Gln	
				210					215					220			
15	CCA	GGT	GGG	GTA	ACA	ATC	ACA	CTG	TTC	TCA	GCC	AAC	ATT	GAT	GCC	ATC	841
	Pro	Gly	Gly	Val	Thr	Ile	Thr	Leu	Phe	Ser	Ala	Asn	Ile	Asp	Ala	Ile	
			225					230					235				
20	ACA	AGC	CTC	AGC	GTT	GGG	GGA	GAG	CTC	GTG	TTT	CAA	ACA	AGC	GTC	CAC	889
	Thr	Ser	Leu	Ser	Val	Gly	Gly	Glu	Leu	Val	Phe	Gln	Thr	Ser	Val	His	
		240					245					250					
25	GGC	CTT	GTA	CTG	GGC	GCC	ACC	ATC	TAC	CTC	ATA	GGC	TTT	GAT	GGG	ACA	937
	Gly	Leu	Val	Leu	Gly	Ala	Thr	Ile	Tyr	Leu	Ile	Gly	Phe	Asp	Gly	Thr	
	255					260					265						
30	ACG	GTA	ATC	ACC	AGG	GCT	GTG	GCC	GCA	AAC	AAT	GGG	CTG	ACG	ACC	GGC	985
	Thr	Val	Ile	Thr	Arg	Ala	Val	Ala	Ala	Asn	Asn	Gly	Leu	Thr	Thr	Gly	
	270				275					280				285			
35	ACC	GAC	AAC	CTT	ATG	CCA	TTC	AAT	CTT	GTG	ATT	CCA	ACA	AAC	GAG	ATA	1033
	Thr	Asp	Asn	Leu	Met	Pro	Phe	Asn	Leu	Val	Ile	Pro	Thr	Asn	Glu	Ile	
			290						295					300			
40	ACC	CAG	CCA	ATC	ACA	TCC	ATC	AAA	CTG	GAG	ATA	GTG	ACC	TCC	AAA	AGT	1081
	Thr	Gln	Pro	Ile	Thr	Ser	Ile	Lys	Leu	Glu	Ile	Val	Thr	Ser	Lys	Ser	
			305					310					315				
45	GGT	GGT	CAG	GCA	GGG	GAT	CAG	ATG	TCA	TGG	TCG	GCA	AGA	GGG	AGC	CTA	1129
	Gly	Gly	Gln	Ala	Gly	Asp	Gln	Met	Ser	Trp	Ser	Ala	Arg	Gly	Ser	Leu	
		320				325						330					
50	GCA	GTG	ACG	ATC	CAT	GGT	GGC	AAC	TAT	CCA	GGG	GCC	CTC	CGT	CCC	GTC	1177
	Ala	Val	Thr	Ile	His	Gly	Gly	Asn	Tyr	Pro	Gly	Ala	Leu	Arg	Pro	Val	
	335					340					345						
55	ACG	CTA	GTG	GCC	TAC	GAA	AGA	GTG	GCA	ACA	GGA	TCC	GTC	GTT	ACG	GTC	1225
	Thr	Leu	Val	Ala	Tyr	Glu	Arg	Val	Ala	Thr	Gly	Ser	Val	Val	Thr	Val	
	350					355				360				365			
60	GCT	GGG	GTG	AGC	AAC	TTC	GAG	CTG	ATC	CCA	AAT	CCT	GAA	CTA	GCA	AAG	1273

	Ala	Gly	Val	Ser	Asn	Phe	Glu	Leu	Ile	Pro	Asn	Pro	Glu	Leu	Ala	Lys	
					370					375					380		
5	AAC	CTG	GTT	ACA	GAA	TAC	GGC	CGA	TTT	GAC	CCA	GGA	GCC	ATG	AAC	TAC	1321
	Asn	Leu	Val	Thr	Glu	Tyr	Gly	Arg	Phe	Asp	Pro	Gly	Ala	Met	Asn	Tyr	
					385				390					395			
10	ACA	AAA	TTG	ATA	CTG	AGT	GAG	AGG	GAC	CGT	CTT	GGC	ATC	AAG	ACC	GTC	1369
	Thr	Lys	Leu	Ile	Leu	Ser	Glu	Arg	Asp	Arg	Leu	Gly	Ile	Lys	Thr	Val	
			400					405					410				
	TGG	CCA	ACA	AGG	GAG	TAC	ACT	GAC	TTT	CGT	GAA	TAC	TTC	ATG	GAG	GTG	1417
	Trp	Pro	Thr	Arg	Glu	Tyr	Thr	Asp	Phe	Arg	Glu	Tyr	Phe	Met	Glu	Val	
		415					420					425					
15	GCC	GAC	CTC	AAC	TCT	CCC	CTG	AAG	ATT	GCA	GGA	GCA	TTC	GGC	TTC	AAA	1465
	Ala	Asp	Leu	Asn	Ser	Pro	Leu	Lys	Ile	Ala	Gly	Ala	Phe	Gly	Phe	Lys	
	430					435				440						445	
20	GAC	ATA	ATC	CGG	GCC	ATA	AGG	AGG	ATA	GCT	GTG	CCG	GTG	GTC	TCC	ACA	1513
	Asp	Ile	Ile	Arg	Ala	Ile	Arg	Arg	Ile	Ala	Val	Pro	Val	Val	Ser	Thr	
					450				455						460		
25	TTG	TTC	CCA	CCT	GCC	GCT	CCC	CTA	GCC	CAT	GCA	ATT	GGG	GAA	GGT	GTA	1561
	Leu	Phe	Pro	Pro	Ala	Ala	Pro	Leu	Ala	His	Ala	Ile	Gly	Glu	Gly	Val	
				465				470					475				
30	GAC	TAC	CTG	CTG	GGC	GAT	GAG	GCA	CAG	GCT	GCT	TCA	GGA	ACT	GCT	CGA	1609
	Asp	Tyr	Leu	Leu	Gly	Asp	Glu	Ala	Gln	Ala	Ala	Ser	Gly	Thr	Ala	Arg	
			480					485					490				
35	GCC	GCG	TCA	GGA	AAA	GCA	AGA	GCT	GCC	TCA	GGC	CGC	ATA	AGG	CAG	CTG	1657
	Ala	Ala	Ser	Gly	Lys	Ala	Arg	Ala	Ala	Ser	Gly	Arg	Ile	Arg	Gln	Leu	
		495					500					505					
	ACT	CTC	GCC	GCC	GAC	AAG	GGG	TAC	GAG	GTA	GTC	GCG	AAT	CTA	TTC	CAG	1705
	Thr	Leu	Ala	Ala	Asp	Lys	Gly	Tyr	Glu	Val	Val	Ala	Asn	Leu	Phe	Gln	
	510					515					520					525	
40	GTG	CCC	CAG	AAT	CCC	GTA	GTC	GAC	GGG	ATT	CTT	GCT	TCA	CCT	GGG	GTA	1753
	Val	Pro	Gln	Asn	Pro	Val	Val	Asp	Gly	Ile	Leu	Ala	Ser	Pro	Gly	Val	
					530					535					540		
45	CTC	CGC	GGT	GCA	CAC	AAC	CTC	GAC	TGC	GTG	TTA	AGA	GAG	GGT	GCC	ACG	1801
	Leu	Arg	Gly	Ala	His	Asn	Leu	Asp	Cys	Val	Leu	Arg	Glu	Gly	Ala	Thr	
				545					550					555			
	CTA	TTC	CCT	GTG	GTT	ATT	ACG	ACA	GTG	GAA	GAC	GCC	ATG	ACA	CCC	AAA	1849

	Leu Phe Pro Val Val Ile Thr Thr Val Glu Asp Ala Met Thr Pro Lys	
	560 565 570	
5	GCA TTG AAC AGC AAA ATG TTT GCT GTC ATT GAA GGC GTG CGA GAA GAC Ala Leu Asn Ser Lys Met Phe Ala Val Ile Glu Gly Val Arg Glu Asp	1897
	575 580 585	
10	CTC CAA CCT CCA TCT CAA AGA GGA TCC TTC ATA CGA ACT CTC TCT GGA Leu Gln Pro Pro Ser Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly	1945
	590 595 600 605	
15	CAC AGA GTC TAT GGA TAT GCT CCA GAT GGG GTA CTT CCA CTG GAG ACT His Arg Val Tyr Gly Tyr Ala Pro Asp Gly Val Leu Pro Leu Glu Thr	1993
	610 615 620	
20	GGG AGA GAC TAC ACC GTT GTC CCA ATA GAT GAT GTC TGG GAC GAC AGC Gly Arg Asp Tyr Thr Val Val Pro Ile Asp Asp Val Trp Asp Asp Ser	2041
	625 630 635	
25	ATT ATG CTG TCC AAA GAT CCC ATA CCT CCT ATT GTG GGA AAC AGT GGA Ile Met Leu Ser Lys Asp Pro Ile Pro Pro Ile Val Gly Asn Ser Gly	2089
	640 645 650	
30	AAT CTA GCC ATA GCT TAC ATG GAT GTG TTT CGA CCC AAA GTC CCA ATC Asn Leu Ala Ile Ala Tyr Met Asp Val Phe Arg Pro Lys Val Pro Ile	2137
	655 660 665	
35	CAT GTG GCT ATG ACG GGA GCC CTC AAT GCT TGT GGC GAG ATT GAG AAA His Val Ala Met Thr Gly Ala Leu Asn Ala Cys Gly Glu Ile Glu Lys	2185
	670 675 680 685	
40	GTA AGC TTT AGA AGC ACC AAG CTC GCC ACT GCA CAC CGA CTT GGC CTT Val Ser Phe Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu Gly Leu	2233
	690 695 700	
45	AGG TTG GCT GGT CCC GGA GCA TTC GAT GTA AAC ACC GGG CCC AAC TGG Arg Leu Ala Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp	2281
	705 710 715	
50	GCA ACG TTC ATC AAA CGT TTC CCT CAC AAT CCA CGC GAC TGG GAC AGG Ala Thr Phe Ile Lys Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg	2329
	720 725 730	
55	CTC CCC TAC CTC AAC CTA CCA TAC CTT CCA CCC AAT GCA GGA CGC CAG Leu Pro Tyr Leu Asn Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln	2377
	735 740 745	
60	TAC CAC CTT GCC ATG GCT GCA TCA GAG TTC AAA GAG ACC CCC GAA CTC	2425

	Tyr	His	Leu	Ala	Met	Ala	Ala	Ser	Glu	Phe	Lys	Glu	Thr	Pro	Glu	Leu	
	750					755					760					765	
5	GAG	AGT	GCC	GTC	AGA	GCA	ATG	GAA	GCA	GCA	GCC	AAC	GTG	GAC	CCA	CTA	2473
	Glu	Ser	Ala	Val	Arg	Ala	Met	Glu	Ala	Ala	Ala	Asn	Val	Asp	Pro	Leu	
					770					775					780		
10	TTC	CAA	TCT	GCA	CTC	AGT	GTG	TTC	ATG	TGG	CTG	GAA	GAG	AAT	GGG	ATT	2521
	Phe	Gln	Ser	Ala	Leu	Ser	Val	Phe	Met	Trp	Leu	Glu	Glu	Asn	Gly	Ile	
					785					790					795		
15	GTG	ACT	GAC	ATG	GCC	AAC	TTC	GCA	CTC	AGC	GAC	CCG	AAC	GCC	CAT	CGG	2569
	Val	Thr	Asp	Met	Ala	Asn	Phe	Ala	Leu	Ser	Asp	Pro	Asn	Ala	His	Arg	
			800						805					810			
20	ATG	CGA	AAT	TTT	CTT	GCA	AAC	GCA	CCA	CAA	GCA	GGC	AGC	AAG	TCG	CAA	2617
	Met	Arg	Asn	Phe	Leu	Ala	Asn	Ala	Pro	Gln	Ala	Gly	Ser	Lys	Ser	Gln	
			815					820					825				
25	AGG	GCC	AAG	TAC	GGG	ACA	GCA	GGC	TAC	GGA	GTG	GAG	GCT	CGG	GGC	CCC	2665
	Arg	Ala	Lys	Tyr	Gly	Thr	Ala	Gly	Tyr	Gly	Val	Glu	Ala	Arg	Gly	Pro	
	830					835					840				845		
30	ACA	CCA	GAG	GAA	GCA	CAG	AGG	GAA	AAA	GAC	ACA	CGG	ATC	TCA	AAG	AAG	2713
	Thr	Pro	Glu	Glu	Ala	Gln	Arg	Glu	Lys	Asp	Thr	Arg	Ile	Ser	Lys	Lys	
					850					855				860			
35	ATG	GAG	ACC	ATG	GGC	ATC	TAC	TTT	GCA	ACA	CCA	GAA	TGG	GTA	GCA	CTC	2761
	Met	Glu	Thr	Met	Gly	Ile	Tyr	Phe	Ala	Thr	Pro	Glu	Trp	Val	Ala	Leu	
				865					870					875			
40	AAT	GGG	CAC	CGA	GGG	CCA	AGC	CCC	GGC	CAG	CTA	AAG	TAC	TGG	CAG	AAC	2809
	Asn	Gly	His	Arg	Gly	Pro	Ser	Pro	Gly	Gln	Leu	Lys	Tyr	Trp	Gln	Asn	
			880					885					890				
45	ACA	CGA	GAA	ATA	CCG	GAC	CCA	AAC	GAG	GAC	TAT	CTA	GAC	TAC	GTG	CAT	2857
	Thr	Arg	Glu	Ile	Pro	Asp	Pro	Asn	Glu	Asp	Tyr	Leu	Asp	Tyr	Val	His	
			895					900				905					
50	GCA	GAG	AAG	AGC	CGG	TTG	GCA	TCA	GAA	GAA	CAA	ATC	CTA	AGG	GCA	GCT	2905
	Ala	Glu	Lys	Ser	Arg	Leu	Ala	Ser	Glu	Glu	Gln	Ile	Leu	Arg	Ala	Ala	
	910					915					920				925		
55	ACG	TCG	ATC	TAC	GGG	GCT	CCA	GGA	CAG	GCA	GAG	CCA	CCC	CAA	GCT	TTC	2953
	Thr	Ser	Ile	Tyr	Gly	Ala	Pro	Gly	Gln	Ala	Glu	Pro	Pro	Gln	Ala	Phe	
					930					935					940		
60	ATA	GAC	GAA	GTT	GCC	AAA	GTC	TAT	GAA	ATC	AAC	CAT	GGA	CGT	GGC	CCA	3001

Ile Asp Glu Val Ala Lys Val Tyr Glu Ile Asn His Gly Arg Gly Pro
 945 950 955

AAC CAA GAA CAG ATG AAA GAT CTG CTC TTG ACT GCG ATG GAG ATG AAG 3049

5 Asn Gln Glu Gln Met Lys Asp Leu Leu Leu Thr Ala Met Glu Met Lys
 960 965 970

CAT CGC AAT CCC AGG CGG GCT CTA CCA AAG CCC AAG CCA AAA CCC AAT 3097

10 His Arg Asn Pro Arg Arg Ala Leu Pro Lys Pro Lys Pro Lys Pro Asn
 975 980 985

GCT CCA ACA CAG AGA CCC CCT GGT CGG CTG GGC CGC TGG ATC AGG ACC 3145

Ala Pro Thr Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr
 990 995 1000 1005

15 GTC TCT GAT GAG GAC CTT GAG TGAGGCTCCT GGGAGTCTCC CGACACCACC 3196

Val Ser Asp Glu Asp Leu Glu
 1010

20 CGCGCAGGTG TGGACACCAA TTCGGCCTTA CAACATCCCA AATTGGATCC GTTCGCGGGT 3256

CCCCT 3261

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1012 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

35 Met Thr Asn Leu Gln Asp Gln Thr Gln Gln Ile Val Pro Phe Ile Arg
 1 5 10 15

40 Ser Leu Leu Met Pro Thr Thr Gly Pro Ala Ser Ile Pro Asp Asp Thr
 20 25 30

Leu Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr Asn Leu Thr
 35 40 45

45 Val Gly Asp Thr Gly Ser Gly Leu Ile Val Phe Phe Pro Gly Phe Pro
 50 55 60

Gly Ser Ile Val Gly Ala His Tyr Thr Leu Gln Gly Asn Gly Asn Tyr

	65		70		75		80									
	Lys	Phe	Asp	Gln	Met	Leu	Leu	Thr	Ala	Gln	Asn	Leu	Pro	Ala	Ser	Tyr
				85						90					95	
5	Asn	Tyr	Cys	Arg	Leu	Val	Ser	Arg	Ser	Leu	Thr	Val	Arg	Ser	Ser	Thr
				100					105					110		
10	Leu	Pro	Gly	Gly	Val	Tyr	Ala	Leu	Asn	Gly	Thr	Ile	Asn	Ala	Val	Thr
			115					120					125			
	Phe	Gln	Gly	Ser	Leu	Ser	Glu	Leu	Thr	Asp	Val	Ser	Tyr	Asn	Gly	Leu
		130					135						140			
15	Met	Ser	Ala	Thr	Ala	Asn	Ile	Asn	Asp	Lys	Ile	Gly	Asn	Val	Leu	Val
	145					150					155				160	
	Gly	Glu	Gly	Val	Thr	Val	Leu	Ser	Leu	Pro	Thr	Ser	Tyr	Asp	Leu	Gly
				165						170				175		
20	Tyr	Val	Arg	Leu	Gly	Asp	Pro	Ile	Pro	Ala	Ile	Gly	Leu	Asp	Pro	Lys
				180					185					190		
25	Met	Val	Ala	Thr	Cys	Asp	Ser	Ser	Asp	Arg	Pro	Arg	Val	Tyr	Thr	Ile
		195						200					205			
	Thr	Ala	Ala	Asp	Asp	Tyr	Gln	Phe	Ser	Ser	Gln	Tyr	Gln	Pro	Gly	Gly
		210					215					220				
30	Val	Thr	Ile	Thr	Leu	Phe	Ser	Ala	Asn	Ile	Asp	Ala	Ile	Thr	Ser	Leu
	225					230					235				240	
	Ser	Val	Gly	Gly	Glu	Leu	Val	Phe	Gln	Thr	Ser	Val	His	Gly	Leu	Val
				245					250					255		
35	Leu	Gly	Ala	Thr	Ile	Tyr	Leu	Ile	Gly	Phe	Asp	Gly	Thr	Thr	Val	Ile
			260						265					270		
40	Thr	Arg	Ala	Val	Ala	Ala	Asn	Asn	Gly	Leu	Thr	Thr	Gly	Thr	Asp	Asn
		275					280						285			
	Leu	Met	Pro	Phe	Asn	Leu	Val	Ile	Pro	Thr	Asn	Glu	Ile	Thr	Gln	Pro
		290					295					300				
45	Ile	Thr	Ser	Ile	Lys	Leu	Glu	Ile	Val	Thr	Ser	Lys	Ser	Gly	Gly	Gln
	305					310					315				320	
	Ala	Gly	Asp	Gln	Met	Ser	Trp	Ser	Ala	Arg	Gly	Ser	Leu	Ala	Val	Thr

					325					330					335	
	Ile	His	Gly	Gly	Asn	Tyr	Pro	Gly	Ala	Leu	Arg	Pro	Val	Thr	Leu	Val
					340				345					350		
5																
	Ala	Tyr	Glu	Arg	Val	Ala	Thr	Gly	Ser	Val	Val	Thr	Val	Ala	Gly	Val
			355					360					365			
10	Ser	Asn	Phe	Glu	Leu	Ile	Pro	Asn	Pro	Glu	Leu	Ala	Lys	Asn	Leu	Val
		370					375					380				
	Thr	Glu	Tyr	Gly	Arg	Phe	Asp	Pro	Gly	Ala	Met	Asn	Tyr	Thr	Lys	Leu
	385					390					395					400
15	Ile	Leu	Ser	Glu	Arg	Asp	Arg	Leu	Gly	Ile	Lys	Thr	Val	Trp	Pro	Thr
					405					410					415	
	Arg	Glu	Tyr	Thr	Asp	Phe	Arg	Glu	Tyr	Phe	Met	Glu	Val	Ala	Asp	Leu
				420					425					430		
20																
	Asn	Ser	Pro	Leu	Lys	Ile	Ala	Gly	Ala	Phe	Gly	Phe	Lys	Asp	Ile	Ile
			435					440					445			
	Arg	Ala	Ile	Arg	Arg	Ile	Ala	Val	Pro	Val	Val	Ser	Thr	Leu	Phe	Pro
25		450					455					460				
	Pro	Ala	Ala	Pro	Leu	Ala	His	Ala	Ile	Gly	Glu	Gly	Val	Asp	Tyr	Leu
	465					470					475					480
30	Leu	Gly	Asp	Glu	Ala	Gln	Ala	Ala	Ser	Gly	Thr	Ala	Arg	Ala	Ala	Ser
					485					490					495	
	Gly	Lys	Ala	Arg	Ala	Ala	Ser	Gly	Arg	Ile	Arg	Gln	Leu	Thr	Leu	Ala
			500						505					510		
35																
	Ala	Asp	Lys	Gly	Tyr	Glu	Val	Val	Ala	Asn	Leu	Phe	Gln	Val	Pro	Gln
			515						520				525			
	Asn	Pro	Val	Val	Asp	Gly	Ile	Leu								

580

585

590

Pro Ser Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val
 595 600 605

5

Tyr Gly Tyr Ala Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp
 610 615 620

10

Tyr Thr Val Val Pro Ile Asp Asp Val Trp Asp Asp Ser Ile Met Leu
 625 630 635 640

Ser Lys Asp Pro Ile Pro Pro Ile Val Gly Asn Ser Gly Asn Leu Ala
 645 650 655

15

Ile Ala Tyr Met Asp Val Phe Arg Pro Lys Val Pro Ile His Val Ala
 660 665 670

Met Thr Gly Ala Leu Asn Ala Cys Gly Glu Ile Glu Lys Val Ser Phe
 675 680 685

20

Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu Gly Leu Arg Leu Ala
 690 695 700

25

Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp Ala Thr Phe
 705 710 715 720

Ile Lys Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg Leu Pro Tyr
 725 730 735

30

Leu Asn Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln Tyr His Leu
 740 745 750

Ala Met Ala Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala
 755 760 765

35

Val Arg Ala Met Glu Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser
 770 775 780

40

Ala Leu Ser Val Phe Met Trp Leu Glu Glu Asn Gly Ile Val Thr Asp
 785 790 795 800

Met Ala Asn Phe Ala Leu Ser Asp Pro Asn Ala His Arg Met Arg Asn
 805 810 815

45

Phe Leu Ala Asn Ala Pro Gln Ala Gly Ser Lys Ser Gln Arg Ala Lys
 820 825 830

Tyr Gly Thr Ala Gly Tyr Gly Val Glu Ala Arg Gly Pro Thr Pro Glu

835 840 845
 Glu Ala Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys Met Glu Thr
 850 855 860
 5 Met Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His
 865 870 875 880
 Arg Gly Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu
 10 885 890 895
 Ile Pro Asp Pro Asn Glu Asp Tyr Leu Asp Tyr Val His Ala Glu Lys
 900 905 910
 15 Ser Arg Leu Ala Ser Glu Glu Gln Ile Leu Arg Ala Ala Thr Ser Ile
 915 920 925
 Tyr Gly Ala Pro Gly Gln Ala Glu Pro Pro Gln Ala Phe Ile Asp Glu
 930 935 940
 20 Val Ala Lys Val Tyr Glu Ile Asn His Gly Arg Gly Pro Asn Gln Glu
 945 950 955 960
 Gln Met Lys Asp Leu Leu Leu Thr Ala Met Glu Met Lys His Arg Asn
 25 965 970 975
 Pro Arg Arg Ala Leu Pro Lys Pro Lys Pro Lys Pro Asn Ala Pro Thr
 980 985 990
 30 Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr Val Ser Asp
 995 1000 1005
 Glu Asp Leu Glu
 1010
 35

(2) INFORMATION FOR SEQ ID NO: 7:

- 40 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3261 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 45 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:97..531

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGATACGATC GGTCTGACCC CGGGGGAGTC ACCCGGGGAC AGGCCGTCAA GGCCTTGTTT 60

10 CAGGATGGGA CTCCTCCTTC TACAACGCTA TCATTC GAA GTT AGT TGA GAT CTG 114
 Glu Val Ser * Asp Leu
 1 5

15 ACA AAC GAT CGC AGC GAT GAC AAA CCT GCA AGA TCA AAC CCA ACA GAT 162
 Thr Asn Asp Arg Ser Asp Asp Lys Pro Ala Arg Ser Asn Pro Thr Asp
 10 15 20

20 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2827 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:112..2745

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGATACGATG GGTCTGACCC TCTGGGAGTC ACGAATTAAC GTGGCTACTA GGGGCGATAC 60

40 CCGCCGCTGG CTGCCACGTT AGTGGCTCCT CTTCTTGATG ATTCTGCCAC C ATG AGT 117
 Met Ser
 1

45 GAC ATT TTC AAC AGT CCA CAG GCG CGA AGC ACG ATC TCA GCA GCG TTC 165
 Asp Ile Phe Asn Ser Pro Gln Ala Arg Ser Thr Ile Ser Ala Ala Phe
 5 10 15

GGC ATA AAG CCT ACT GCT GGA CAA GAC GTG GAA GAA CTC TTG ATC CCT 213

	Gly	Ile	Lys	Pro	Thr	Ala	Gly	Gln	Asp	Val	Glu	Glu	Leu	Leu	Ile	Pro	
	20						25				30						
5	AAA	GTT	TGG	GTG	CCA	CCT	GAG	GAT	CCG	CTT	GCC	AGC	CCT	AGT	CGA	CTG	261
	Lys	Val	Trp	Val	Pro	Pro	Glu	Asp	Pro	Leu	Ala	Ser	Pro	Ser	Arg	Leu	
	35					40				45					50		
10	GCA	AAG	TTC	CTC	AGA	GAG	AAC	GGC	TAC	AAA	GTT	TTG	CAG	CCG	CGG	TCT	309
	Ala	Lys	Phe	Leu	Arg	Glu	Asn	Gly	Tyr	Lys	Val	Leu	Gln	Pro	Arg	Ser	
				55					60					65			
15	CTG	CCC	GAG	AAT	GAG	GAG	TAT	GAG	ACC	GAC	CAA	ATA	CTC	CCA	GAC	TTA	357
	Leu	Pro	Glu	Asn	Glu	Glu	Tyr	Glu	Thr	Asp	Gln	Ile	Leu	Pro	Asp	Leu	
				70				75					80				
20	GCA	TGG	ATG	CGA	CAG	ATA	GAA	GGG	GCT	GTT	TTA	AAA	CCC	ACT	CTA	TCT	405
	Ala	Trp	Met	Arg	Gln	Ile	Glu	Gly	Ala	Val	Leu	Lys	Pro	Thr	Leu	Ser	
			85					90					95				
25	CTC	CCT	ATT	GGA	GAT	CAG	GAG	TAC	TTC	CCA	AAG	TAC	TAC	CCA	ACA	CAT	453
	Leu	Pro	Ile	Gly	Asp	Gln	Glu	Tyr	Phe	Pro	Lys	Tyr	Tyr	Pro	Thr	His	
	100					105						110					
30	CGC	CCT	AGC	AAG	GAG	AAG	CCC	AAT	GCG	TAC	CCG	CCA	GAC	ATC	GCA	CTA	501
	Arg	Pro	Ser	Lys	Glu	Lys	Pro	Asn	Ala	Tyr	Pro	Pro	Asp	Ile	Ala	Leu	
	115					120					125				130		
35	CTC	AAG	CAG	ATG	ATT	TAC	CTG	TTT	CTC	CAG	GTT	CCA	GAG	GCC	AAC	GAG	549
	Leu	Lys	Gln	Met	Ile	Tyr	Leu	Phe	Leu	Gln	Val	Pro	Glu	Ala	Asn	Glu	
				135					140						145		
40	GGC	CTA	AAG	GAT	GAA	GTA	ACC	CTC	TTG	ACC	CAA	AAC	ATA	AGG	GAC	AAG	597
	Gly	Leu	Lys	Asp	Glu	Val	Thr	Leu	Leu	Thr	Gln	Asn	Ile	Arg	Asp	Lys	
				150					155					160			
45	GCC	TAT	GGA	AGT	GGG	ACC	TAC	ATG	GGA	CAA	GCA	ACT	CGA	CTT	GTG	GCC	645
	Ala	Tyr	Gly	Ser	Gly	Thr	Tyr	Met	Gly	Gln	Ala	Thr	Arg	Leu	Val	Ala	
			165					170					175				
50	ATG	AAG	GAG	GTC	GCC	ACT	GGA	AGA	AAC	CCA	AAC	AAG	GAT	CCT	CTA	AAG	693
	Met	Lys	Glu	Val	Ala	Thr	Gly	Arg	Asn	Pro	Asn	Lys	Asp	Pro	Leu	Lys	
	180						185					190					
55	CTT	GGG	TAC	ACT	TTT	GAG	AGC	ATC	GCG	CAG	CTA	CTT	GAC	ATC	ACA	CTA	741
	Leu	Gly	Tyr	Thr	Phe	Glu	Ser	Ile	Ala	Gln	Leu	Leu	Asp	Ile	Thr	Leu	
	195					200					205				210		
60	CCG	GTA	GGC	CCA	CCC	GGT	GAG	GAT	GAC	AAG	CCC	TGG	GTG	CCA	CTC	ACA	789

	Pro	Val	Gly	Pro	Pro	Gly	Glu	Asp	Asp	Lys	Pro	Trp	Val	Pro	Leu	Thr	
				215						220					225		
5	AGA	GTG	CCG	TCA	CGG	ATG	TTG	GTG	CTG	ACG	GGA	GAC	GTA	GAT	GGC	GAC	837
	Arg	Val	Pro	Ser	Arg	Met	Leu	Val	Leu	Thr	Gly	Asp	Val	Asp	Gly	Asp	
				230						235					240		
10	TTT	GAG	GTT	GAA	GAT	TAC	CTT	CCC	AAA	ATC	AAC	CTC	AAG	TCA	TCA	AGT	885
	Phe	Glu	Val	Glu	Asp	Tyr	Leu	Pro	Lys	Ile	Asn	Leu	Lys	Ser	Ser	Ser	
				245						250					255		
15	GGA	CTA	CCA	TAT	GTA	GGT	CGC	ACC	AAA	GGA	GAG	ACA	ATT	GGC	GAG	ATG	933
	Gly	Leu	Pro	Tyr	Val	Gly	Arg	Thr	Lys	Gly	Glu	Thr	Ile	Gly	Glu	Met	
				260						265					270		
20	ATA	GCT	ATA	TCA	AAC	CAG	TTT	CTC	AGA	GAG	CTA	TCA	ACA	CTG	TTG	AAG	981
	Ile	Ala	Ile	Ser	Asn	Gln	Phe	Leu	Arg	Glu	Leu	Ser	Thr	Leu	Leu	Lys	
				275						280					285		290
25	CAA	GGT	GCA	GGG	ACA	AAG	GGG	TCA	AAC	AAG	AAG	AAG	CTA	CTC	AGC	ATG	1029
	Gln	Gly	Ala	Gly	Thr	Lys	Gly	Ser	Asn	Lys	Lys	Lys	Leu	Leu	Ser	Met	
						295									300		305
30	TTA	AGT	GAC	TAT	TGG	TAC	TTA	TCA	TGC	GGG	CTT	TTG	TTT	CCA	AAG	GCT	1077
	Leu	Ser	Asp	Tyr	Trp	Tyr	Leu	Ser	Cys	Gly	Leu	Leu	Phe	Pro	Lys	Ala	
				310						315					320		
35	GAA	AGG	TAC	GAC	AAA	AGT	ACA	TGG	CTC	ACC	AAG	ACC	CGG	AAC	ATA	TGG	1125
	Glu	Arg	Tyr	Asp	Lys	Ser	Thr	Trp	Leu	Thr	Lys	Thr	Arg	Asn	Ile	Trp	
				325						330					335		
40	TCA	GCT	CCA	TCC	CCA	ACA	CAC	CTC	ATG	ATC	TCC	ATG	ATC	ACC	TGG	CCC	1173
	Ser	Ala	Pro	Ser	Pro	Thr	His	Leu	Met	Ile	Ser	Met	Ile	Thr	Trp	Pro	
				340						345					350		
45	GTG	ATG	TCC	AAC	AGC	CCA	AAT	AAC	GTG	TTG	AAC	ATT	GAA	GGG	TGT	CCA	1221
	Val	Met	Ser	Asn	Ser	Pro	Asn	Asn	Val	Leu	Asn	Ile	Glu	Gly	Cys	Pro	
				355						360					365		370
50	TCA	CTC	TAC	AAA	TTC	AAC	CCG	TTC	AGA	GGA	GGG	TTG	AAC	AGG	ATC	GTC	1269
	Ser	Leu	Tyr	Lys	Phe	Asn	Pro	Phe	Arg	Gly	Gly	Leu	Asn	Arg	Ile	Val	
					375					380					385		
55	GAG	TGG	ATA	TTG	GCC	CCG	GAA	GAA	CCC	AAG	GCT	CTT	GTA	TAT	GCG	GAC	1317
	Glu	Trp	Ile	Leu	Ala	Pro	Glu	Glu	Pro	Lys	Ala	Leu	Val	Tyr	Ala	Asp	
				390						395					400		
	AAC	ATA	TAC	ATT	GTC	CAC	TCA	AAC	ACG	TGG	TAC	TCA	ATT	GAC	CTA	GAG	1365

	Asn	Ile	Tyr	Ile	Val	His	Ser	Asn	Thr	Trp	Tyr	Ser	Ile	Asp	Leu	Glu	
	405							410					415				
5	AAG	GGT	GAG	GCA	AAC	TGC	ACT	CGC	CAA	CAC	ATG	CAA	GCC	GCA	ATG	TAC	1413
	Lys	Gly	Glu	Ala	Asn	Cys	Thr	Arg	Gln	His	Met	Gln	Ala	Ala	Met	Tyr	
	420						425				430						
10	TAC	ATA	CTC	ACC	AGA	GGG	TGG	TCA	GAC	AAC	GGC	GAC	CCA	ATG	TTC	AAT	1461
	Tyr	Ile	Leu	Thr	Arg	Gly	Trp	Ser	Asp	Asn	Gly	Asp	Pro	Met	Phe	Asn	
	435					440				445					450		
15	CAA	ACA	TGG	GCC	ACC	TTT	GCC	ATG	AAC	ATT	GCC	CCT	GCT	CTA	GTG	GTG	1509
	Gln	Thr	Trp	Ala	Thr	Phe	Ala	Met	Asn	Ile	Ala	Pro	Ala	Leu	Val	Val	
				455					460					465			
20	GAC	TCA	TCG	TGC	CTG	ATA	ATG	AAC	CTG	CAA	ATT	AAG	ACC	TAT	GGT	CAA	1557
	Asp	Ser	Ser	Cys	Leu	Ile	Met	Asn	Leu	Gln	Ile	Lys	Thr	Tyr	Gly	Gln	
				470				475						480			
25	GGC	AGC	GGG	AAT	GCA	GCC	ACG	TTC	ATC	AAC	AAC	CAC	CTC	TTG	AGC	ACG	1605
	Gly	Ser	Gly	Asn	Ala	Ala	Thr	Phe	Ile	Asn	Asn	His	Leu	Leu	Ser	Thr	
		485					490					495					
30	CTA	GTG	CTT	GAC	CAG	TGG	AAC	TTG	ATG	AGA	CAG	CCC	AGA	CCA	GAC	AGC	1653
	Leu	Val	Leu	Asp	Gln	Trp	Asn	Leu	Met	Arg	Gln	Pro	Arg	Pro	Asp	Ser	
	500					505					510						
35	GAG	GAG	TTC	AAA	TCA	ATT	GAG	GAC	AAG	CTA	GGT	ATC	AAC	TTT	AAG	ATT	1701
	Glu	Glu	Phe	Lys	Ser	Ile	Glu	Asp	Lys	Leu	Gly	Ile	Asn	Phe	Lys	Ile	
	515					520				525				530			
40	GAG	AGG	TCC	ATT	GAT	GAT	ATC	AGG	GGC	AAG	CTG	AGA	CAG	CTT	GTC	CTC	1749
	Glu	Arg	Ser	Ile	Asp	Asp	Ile	Arg	Gly	Lys	Leu	Arg	Gln	Leu	Val	Leu	
				535				540					545				
45	CTT	GCA	CAA	CCA	GGG	TAC	CTG	AGT	GGG	GGG	GTT	GAA	CCA	GAA	CAA	TCC	1797
	Leu	Ala	Gln	Pro	Gly	Tyr	Leu	Ser	Gly	Gly	Val	Glu	Pro	Glu	Gln	Ser	
			550					555				560					
50	AGC	CCA	ACT	GTT	GAG	CTT	GAC	CTA	CTA	GGG	TGG	TCA	GCT	ACA	TAC	AGC	1845
	Ser	Pro	Thr	Val	Glu	Leu	Asp	Leu	Leu	Gly	Trp	Ser	Ala	Thr	Tyr	Ser	
		565					570					575					
55	AAA	GAT	CTC	GGG	ATC	TAT	GTG	CCG	GTG	CTT	GAC	AAG	GAA	CGC	CTA	TTT	1893
	Lys	Asp	Leu	Gly	Ile	Tyr	Val	Pro	Val	Leu	Asp	Lys	Glu	Arg	Leu	Phe	
		580					585					590					
60	TGT	TCT	GCT	GCG	TAT	CCC	AAG	GGA	GTA	GAG	AAC	AAG	AGT	CTC	AAG	TCC	1941

	Cys	Ser	Ala	Ala	Tyr	Pro	Lys	Gly	Val	Glu	Asn	Lys	Ser	Leu	Lys	Ser	
	595					600					605					610	
5	AAA	GTC	GGG	ATC	GAG	CAG	GCA	TAC	AAG	GTA	GTC	AGG	TAT	GAG	GCG	TTG	1989
	Lys	Val	Gly	Ile	Glu	Gln	Ala	Tyr	Lys	Val	Val	Arg	Tyr	Glu	Ala	Leu	
					615					620					625		
10	AGG	TTG	GTA	GGT	GGT	TGG	AAC	TAC	CCA	CTC	CTG	AAC	AAA	GCC	TGC	AAG	2037
	Arg	Leu	Val	Gly	Gly	Trp	Asn	Tyr	Pro	Leu	Leu	Asn	Lys	Ala	Cys	Lys	
					630				635					640			
15	AAT	AAC	GCA	GGC	GCC	GCT	CGG	CGG	CAT	CTG	GAG	GCC	AAG	GGG	TTC	CCA	2085
	Asn	Asn	Ala	Gly	Ala	Ala	Arg	Arg	His	Leu	Glu	Ala	Lys	Gly	Phe	Pro	
			645					650					655				
20	CTC	GAC	GAG	TTC	CTA	GCC	GAG	TGG	TCT	GAG	CTG	TCA	GAG	TTC	GGT	GAG	2133
	Leu	Asp	Glu	Phe	Leu	Ala	Glu	Trp	Ser	Glu	Leu	Ser	Glu	Phe	Gly	Glu	
		660					665				670						
25	GCC	TTC	GAA	GGC	TTC	AAT	ATC	AAG	CTG	ACC	GTA	ACA	TCT	GAG	AGC	CTA	2181
	Ala	Phe	Glu	Gly	Phe	Asn	Ile	Lys	Leu	Thr	Val	Thr	Ser	Glu	Ser	Leu	
		675				680				685					690		
30	GCC	GAA	CTG	AAC	AAG	CCA	GTA	CCC	CCC	AAG	CCC	CCA	AAT	GTC	AAC	AGA	2229
	Ala	Glu	Leu	Asn	Lys	Pro	Val	Pro	Pro	Lys	Pro	Pro	Asn	Val	Asn	Arg	
					695				700					705			
35	CCA	GTC	AAC	ACT	GGG	GGA	CTC	AAG	GCA	GTC	AGC	AAC	GCC	CTC	AAG	ACC	2277
	Pro	Val	Asn	Thr	Gly	Gly	Leu	Lys	Ala	Val	Ser	Asn	Ala	Leu	Lys	Thr	
				710				715					720				
40	GGT	CGG	TAC	AGG	AAC	GAA	GCC	GGA	CTG	AGT	GGT	CTC	GTC	CTT	CTA	GCC	2325
	Gly	Arg	Tyr	Arg	Asn	Glu	Ala	Gly	Leu	Ser	Gly	Leu	Val	Leu	Leu	Ala	
			725				730					735					
45	ACA	GCA	AGA	AGC	CGT	CTG	CAA	GAT	GCA	GTT	AAG	GCC	AAG	GCA	GAA	GCC	2373
	Thr	Ala	Arg	Ser	Arg	Leu	Gln	Asp	Ala	Val	Lys	Ala	Lys	Ala	Glu	Ala	
		740				745					750						
50	GAG	AAA	CTC	CAC	AAG	TCC	AAG	CCA	GAC	GAC	CCC	GAT	GCA	GAC	TGG	TTC	2421
	Glu	Lys	Leu	His	Lys	Ser	Lys	Pro	Asp	Asp	Pro	Asp	Ala	Asp	Trp	Phe	
		755				760				765					770		
55	GAA	AGA	TCA	GAA	ACT	CTG	TCA	GAC	CTT	CTG	GAG	AAA	GCC	GAC	ATC	GCC	2469
	Glu	Arg	Ser	Glu	Thr	Leu	Ser	Asp	Leu	Leu	Glu	Lys	Ala	Asp	Ile	Ala	
					775				780					785			
60	AGC	AAG	GTC	GCC	CAC	TCA	GCA	CTC	GTG	GAA	ACA	AGC	GAC	GCC	CTT	GAA	2517

[illegible]

CLAIMS

- 1 A birnavirus mutant which is not able to produce a native VP5 protein as a result of a mutation in the VP5 gene of the birnavirus genome.
- 2 A birnavirus mutant according to claim 1, characterised in that the mutation is a substitution.
- 3 A birnavirus mutant according to claim 1, characterised in that the mutation is an insertion of a heterologous nucleic acid sequence.
- 4 A birnavirus mutant according to claim 3, characterised in that the heterologous nucleic acid sequence encodes a polypeptide and the heterologous nucleic acid sequence is under the control of an expression control sequence regulating the expression of the sequence in a cell infected with the virus mutant.
- 5 A birnavirus mutant according to claims 1-4, characterised in that the birnavirus is infectious bursal disease virus (IBDV).
- 6 A birnavirus mutant according to claim 5, characterised in that the mutation is in the genome of a virulent field virus.
- 7 A birnavirus mutant according to claim 5, characterised in that the mutation is in the genome of vaccine strain, preferably in vaccine strain D78.
- 8 A birnavirus mutant according to claims 5-7, characterised in that the mutant has a mutated start codon and three stop codons in the 5'-end of the VP5 gene as shown in SEQ ID No: 7.
- 9 A birnavirus according to claims 5-8, characterised in that the IBDV expresses a chimeric VP2 protein comprising virus neutralising epitopes of different antigenic IBDV types.

10 A vaccine against a birnavirus infection in animals, characterised in that it comprises a birnavirus mutant according to claims 1-9 and a pharmaceutically acceptable carrier.

11 A method for determining birnavirus infection in an animal, characterised in that a sample of the animal is examined for the presence of anti-VP5 antibodies.

12 A method according to claim 11, characterised in that the method comprises the steps of:

- (i) incubating a sample suspected of containing anti-birnavirus antibodies, with VP5 antigen,
- (ii) allowing the formation of antibody-antigen complex , and
- (ii) detecting the presence of the antibody-antigen complex.

13 A diagnostic test kit suitable for carrying out a method according to claims 11-12.

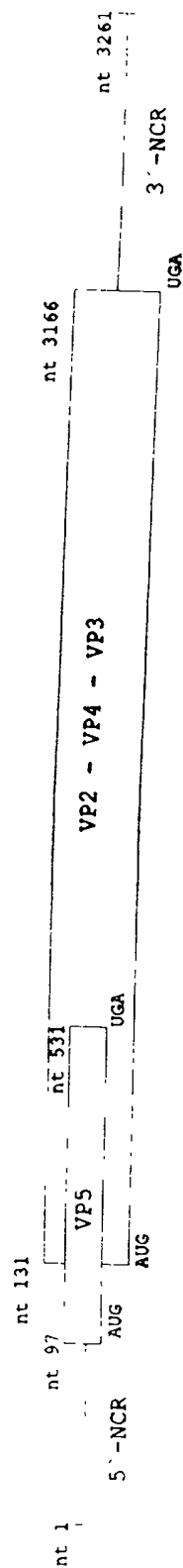
14 Use of the lack of the expression of native VP5 protein by a birnavirus mutant as a marker to distinguish vaccinated animals from animals infected with naturally-occurring birnavirus.

ABSTRACT

5

Genomic organization of segment A of strain D78 and segment B of strain P2

D78 segment A



P2 segment B

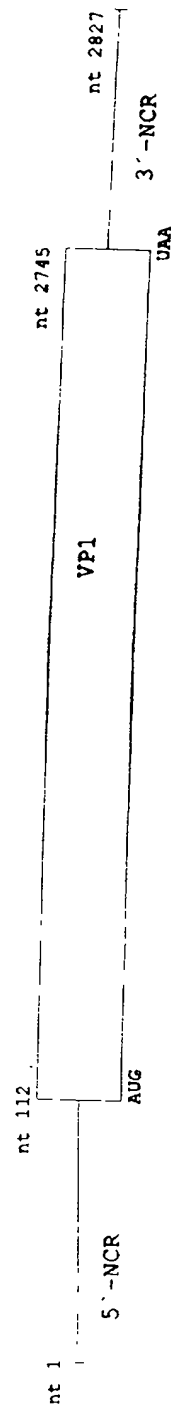
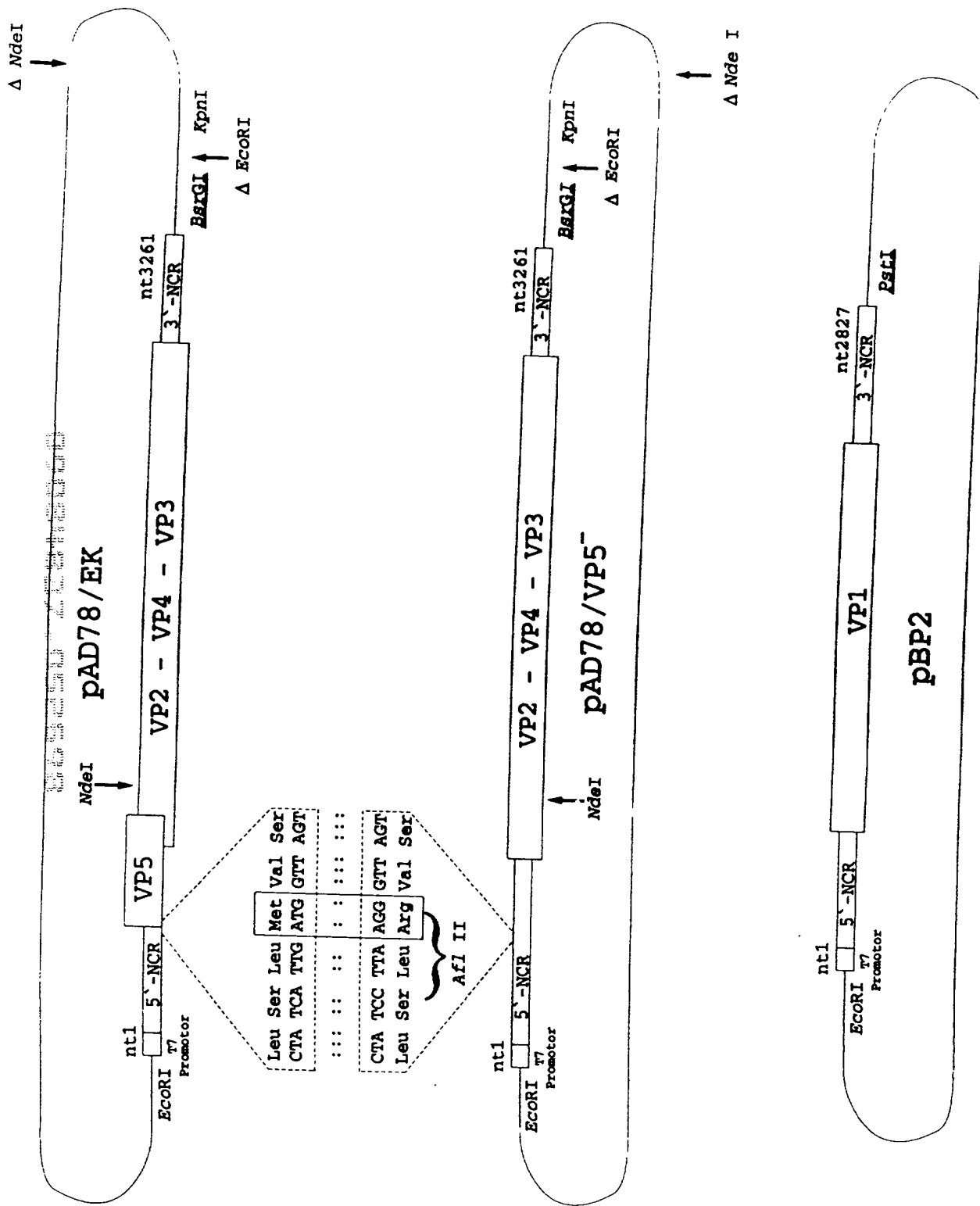


Figure 1

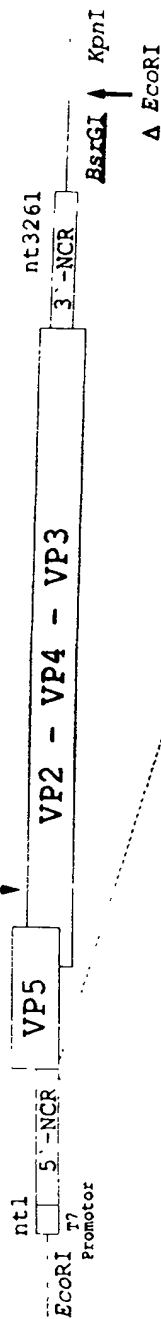
Figure 2



Δ NdeI

↑

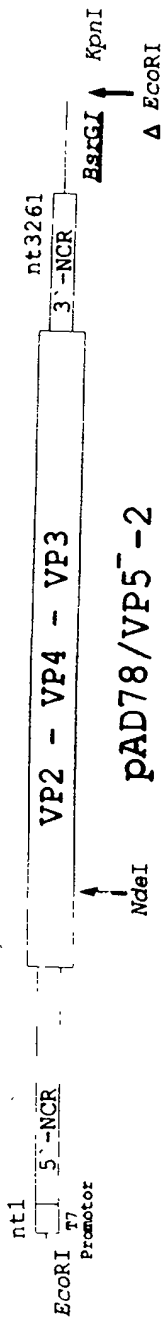
pAD78/EK



Leu	Ser	Leu	Met	Val	Ser	Arg	Asp	Gln	Thr	Asn
CTA	TCA	TTG	ATG	GTT	AGT	AGA	GAT	CAG	ACA	AAC
:::	:::	:::	:::	:::	:::	:::	:::	:::	:::	:::
CTA	TCC	TTC	GAA	GTT	AGT	TGA	GAT	CTG	ACA	AAC
Leu	Ser	Phe	Glu	Val	Ser	*	Asp	Leu	Thr	Asn

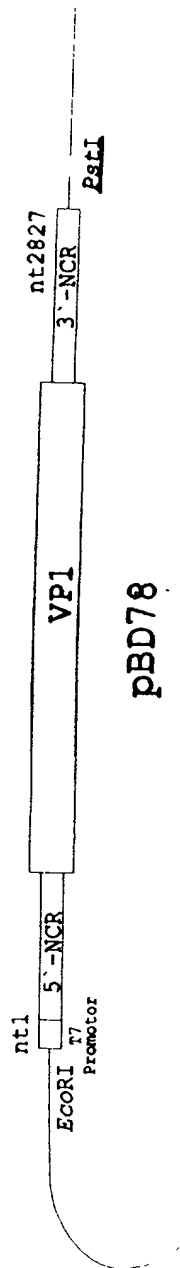
BstBI

pAD78/VP5⁻-2



Δ Nde I

↑



Δ Hind III, Nde I, Xba I, Nar I

Figure 3

Figure 5

